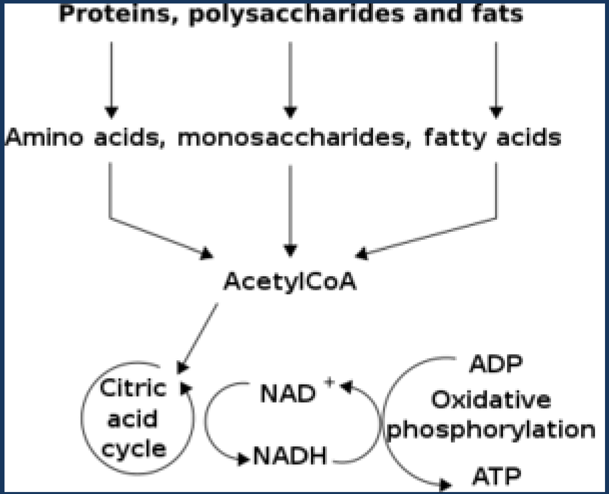
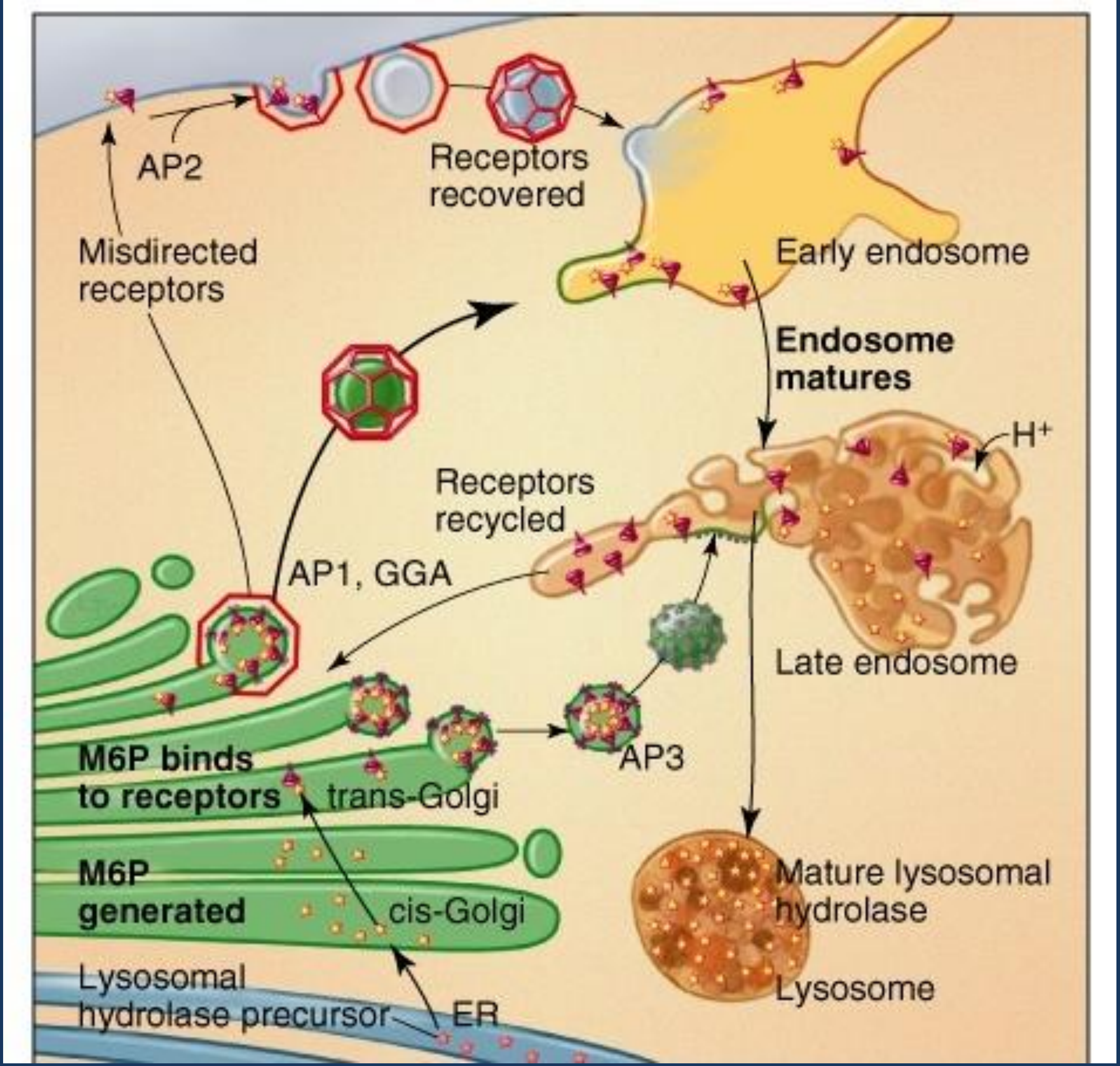
Катаболические процессы у эукариот


Proteins, polysaccharides and fats
Amino acids, monosaccharides, fatty acids
AcetylCoA
Citric acid cycle
NAD +
NADH
ADP
Oxidative phosphorylation
ATP

с/к «Биохимия клетки»
Лекция 2-3

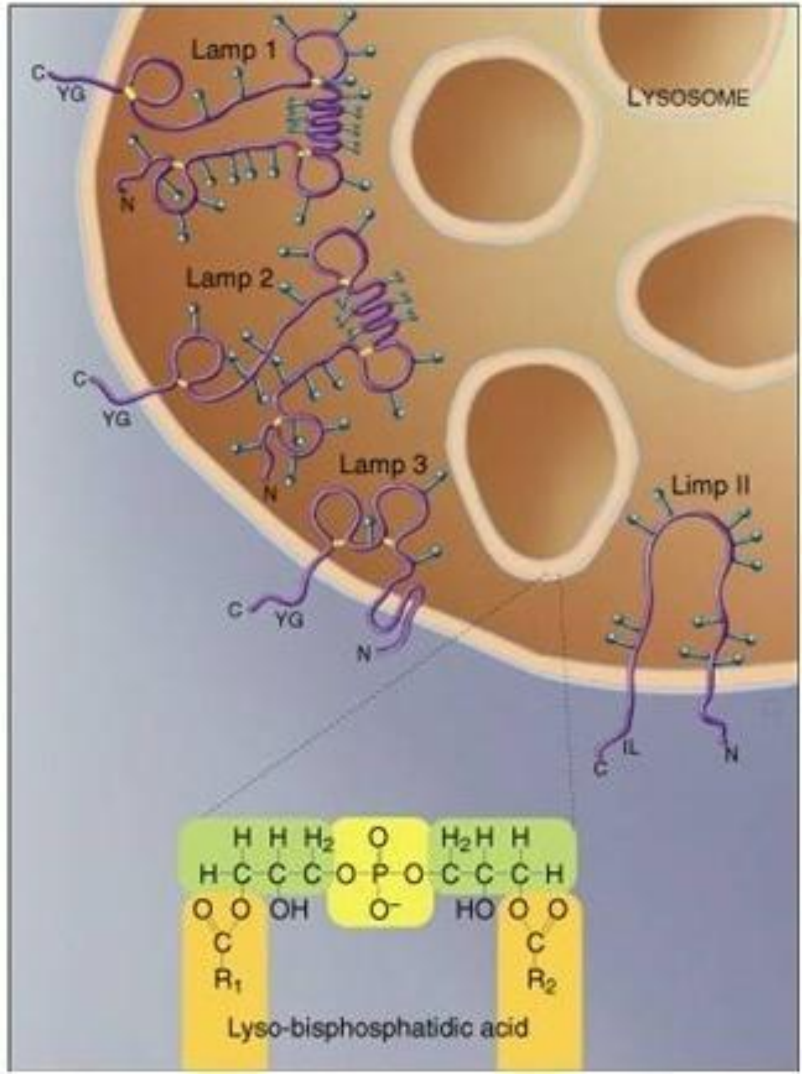
Lysosomes and Endosomes



- Early endosome
  - pH 6.5-6
  - sorting endosome
- Late endosome
  - pH 5.5-5
- Lysosome
  - pH 5-4.5

Structure of Lysosomes

- Protein rich membranes
  - Lamp/Limp family of glycoproteins
- Unusual lipids
  - lyso-bisphosphatidic acid
  - thought to protect lysosomal membrane lipids from action of lumenal lipases
- Multivesicular bodies (late endosomes/lysosomes)
  - invagination of the limiting membrane
  - forms internal membranes
    - TM proteins can segregate into limiting or internal membranes
    - limiting membrane can be recycled, internal is not

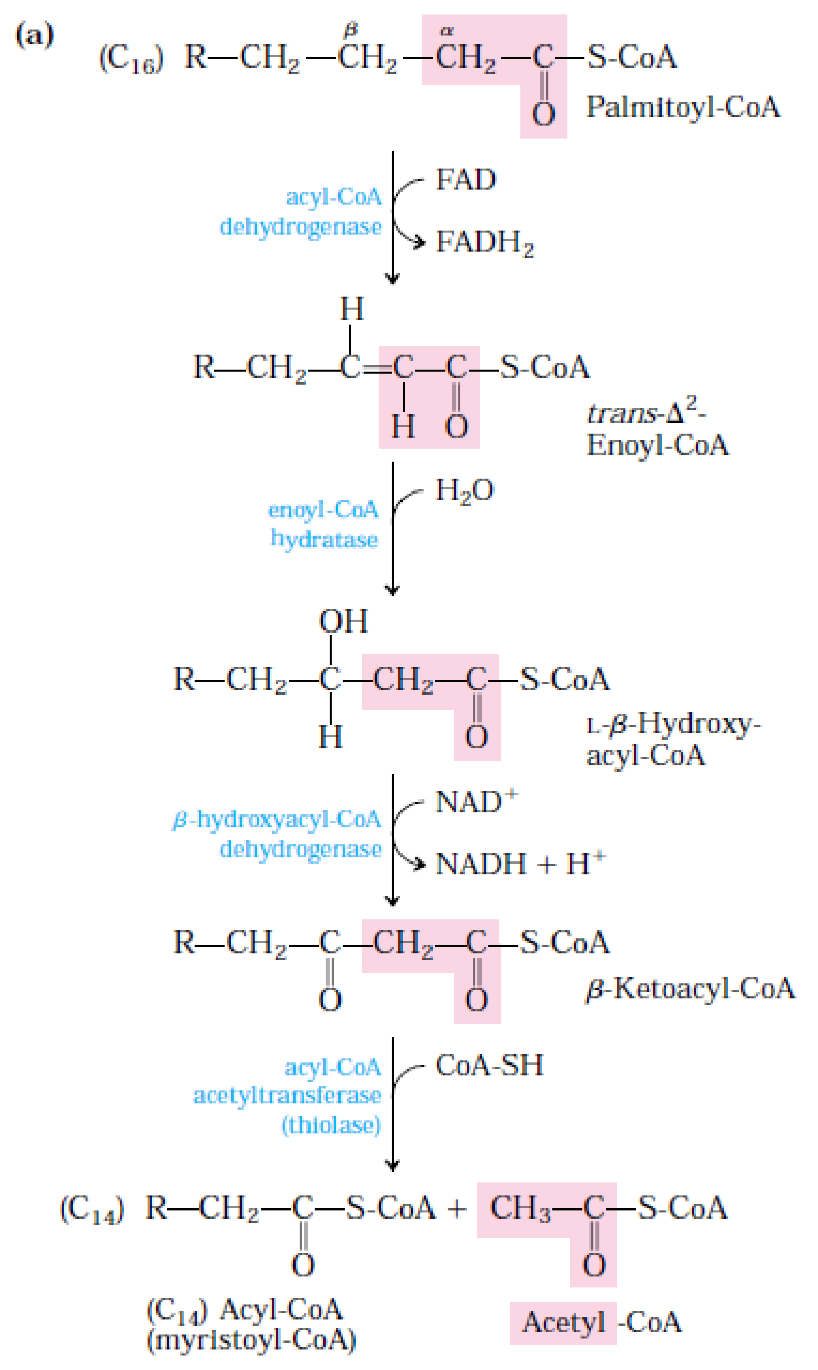


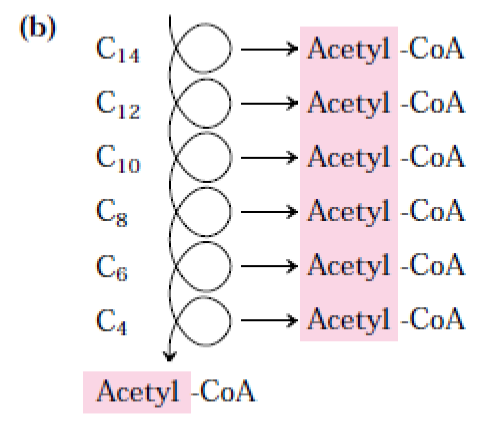
Основные группы лизосомальных гидролаз

Вид катаболизма	Фермент	Субстрат
Деградация НК:	Кислая РНКаза Кислая ДНКаза	РНК ДНК
Деградация углеводов:	Галактозидаза Гликозидаза Лизоцим	Галактозиды Гликоген Бактериальные клеточные стенки
Деградация протеогликанов:	глюкуронидаза, гиалуронидаза	Гетерополисахариды
Деградация белков:	катепсины Коллагеназа	Белки Коллаген
Фосфатазы:	кислая фосфатаза Кислая ФДЭ	Большинство фосфомоноэфиров Олигонуклеотиды
Сульфатаза:	арилсульфатаза	Органические сульфаты

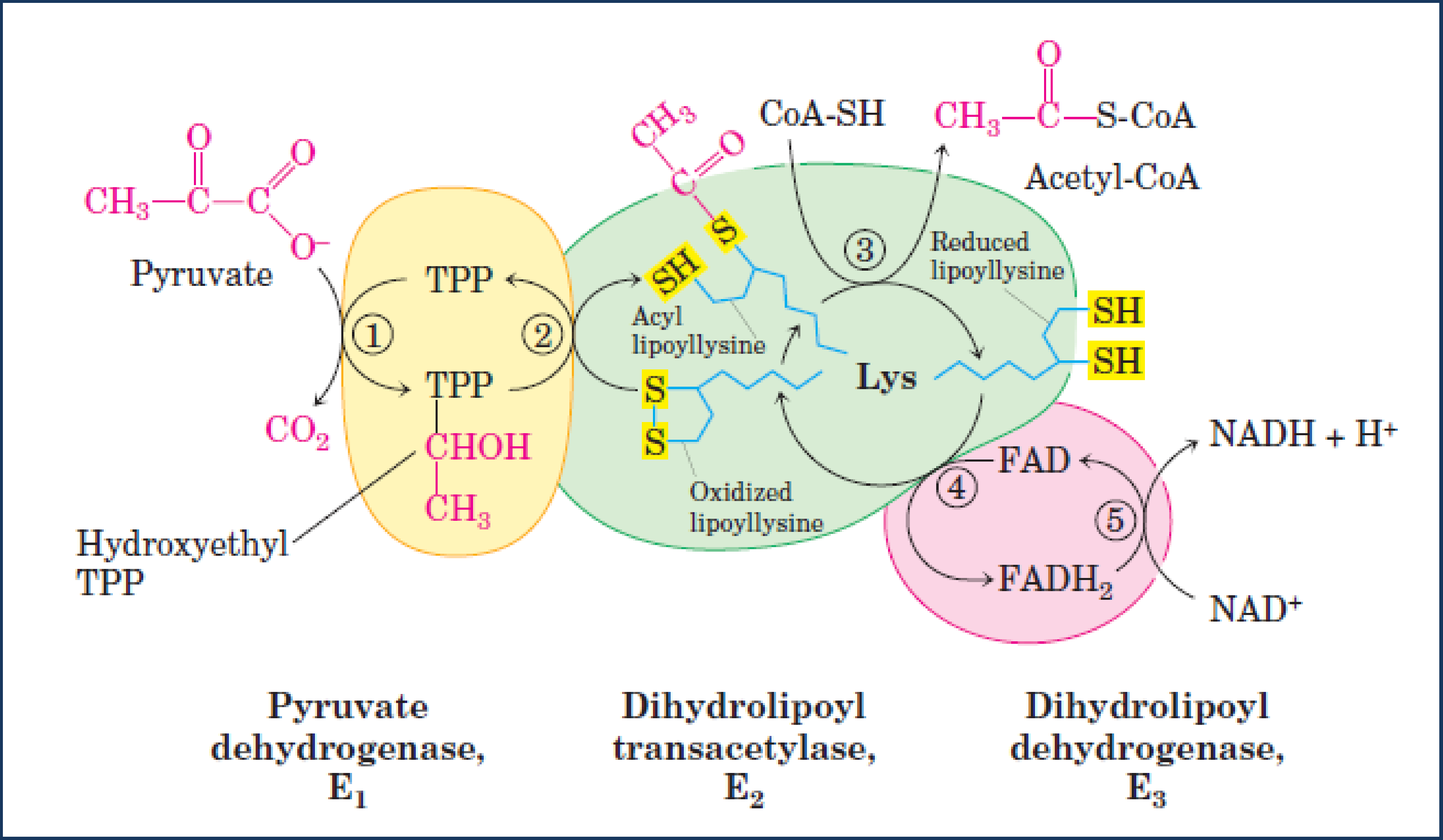
Виды катаболических процессов в митохондриях

- Бета-окисление жирных кислот
- Окислительное декарбоксилирование пирувата
- ЦТК
- Катаболизм аминокислот, реакции синтеза мочевины

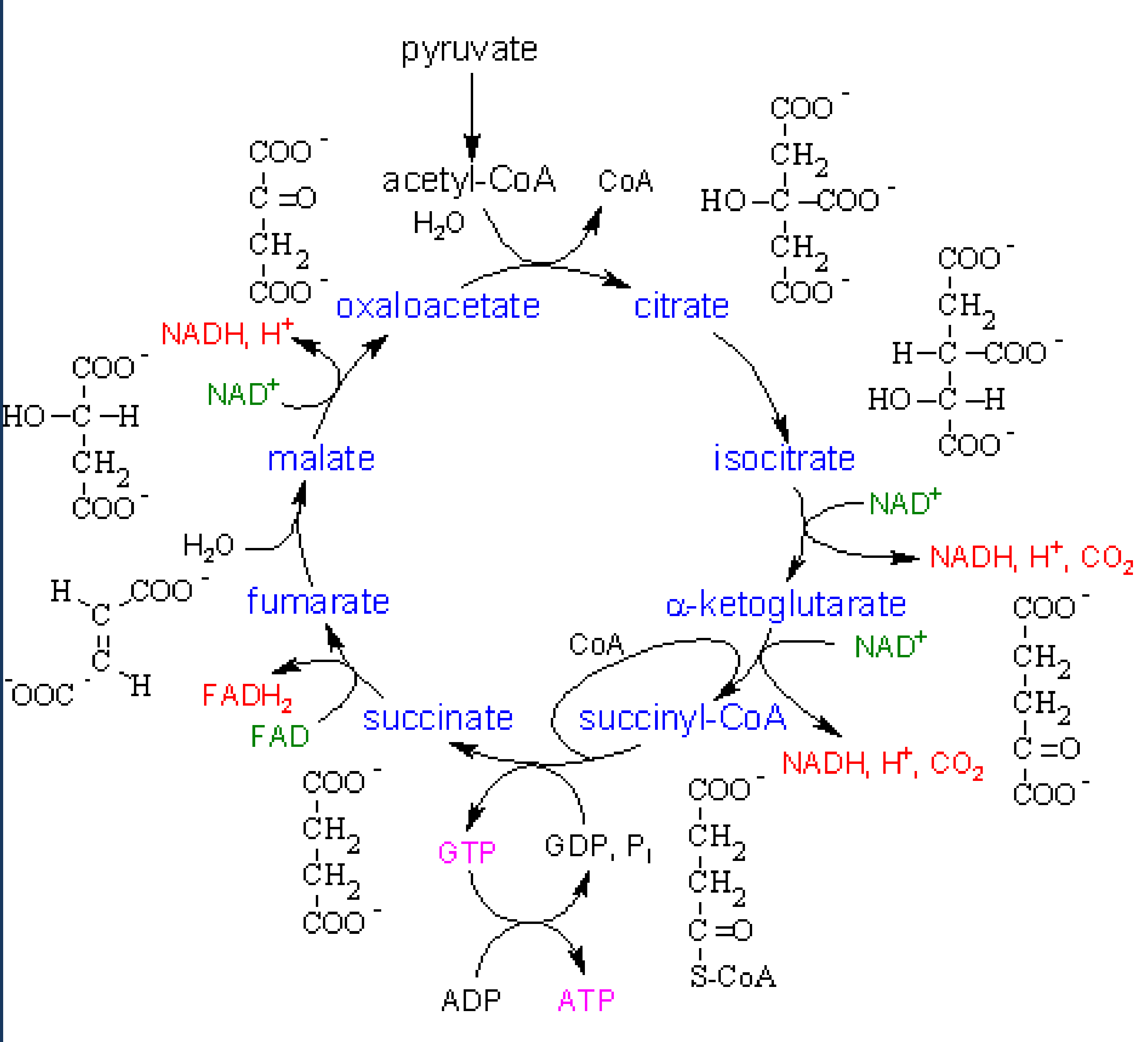

(a)
(C16) R—CH2—CH2—CH2—C—S-CoA
β
α
O
Palmitoyl-CoA
acyl-CoA dehydrogenase
FAD
FADH2
H
R—CH2—C=C—C—S-CoA
H O
trans-Δ2-Enoyl-CoA
enoyl-CoA hydratase
H2O
OH
R—CH2—C—CH2—C—S-CoA
H O
L-β-Hydroxy-acyl-CoA
β-hydroxyacyl-CoA dehydrogenase
NAD+
NADH + H+
R—CH2—C—CH2—C—S-CoA
O O
β-Ketoacyl-CoA
acyl-CoA acetyltransferase (thiolase)
CoA-SH
(C14) R—CH2—C—S-CoA + CH3—C—S-CoA
O O
(C14) Acyl-CoA (myristoyl-CoA)
Acetyl -CoA


(b)
C14
C12
C10
C8
C6
C4
Acetyl -CoA
Acetyl -CoA
Acetyl -CoA
Acetyl -CoA
Acetyl -CoA
Acetyl -CoA
Acetyl -CoA

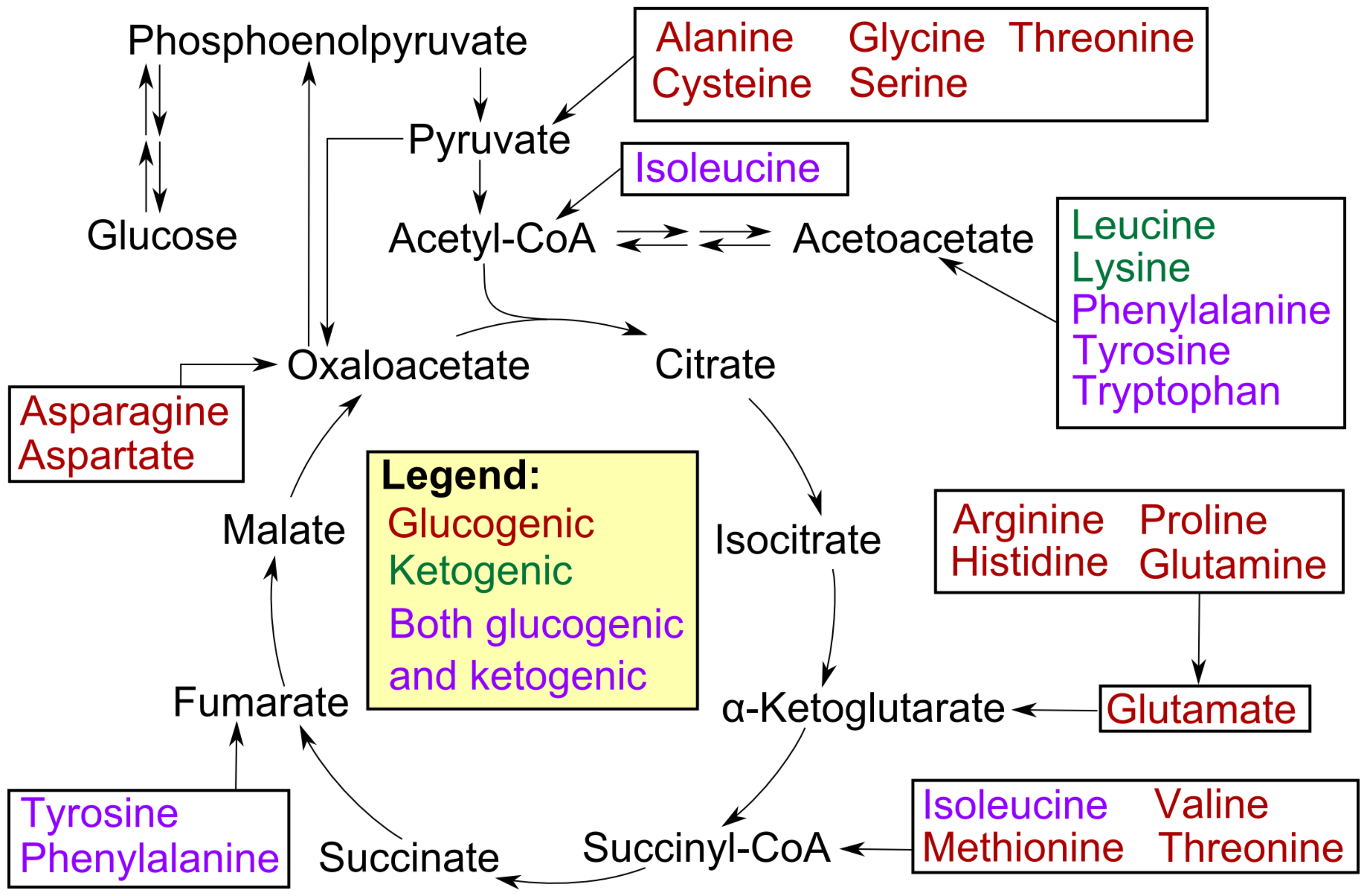
Oxidative decarboxylation of pyruvate to acetyl-CoA

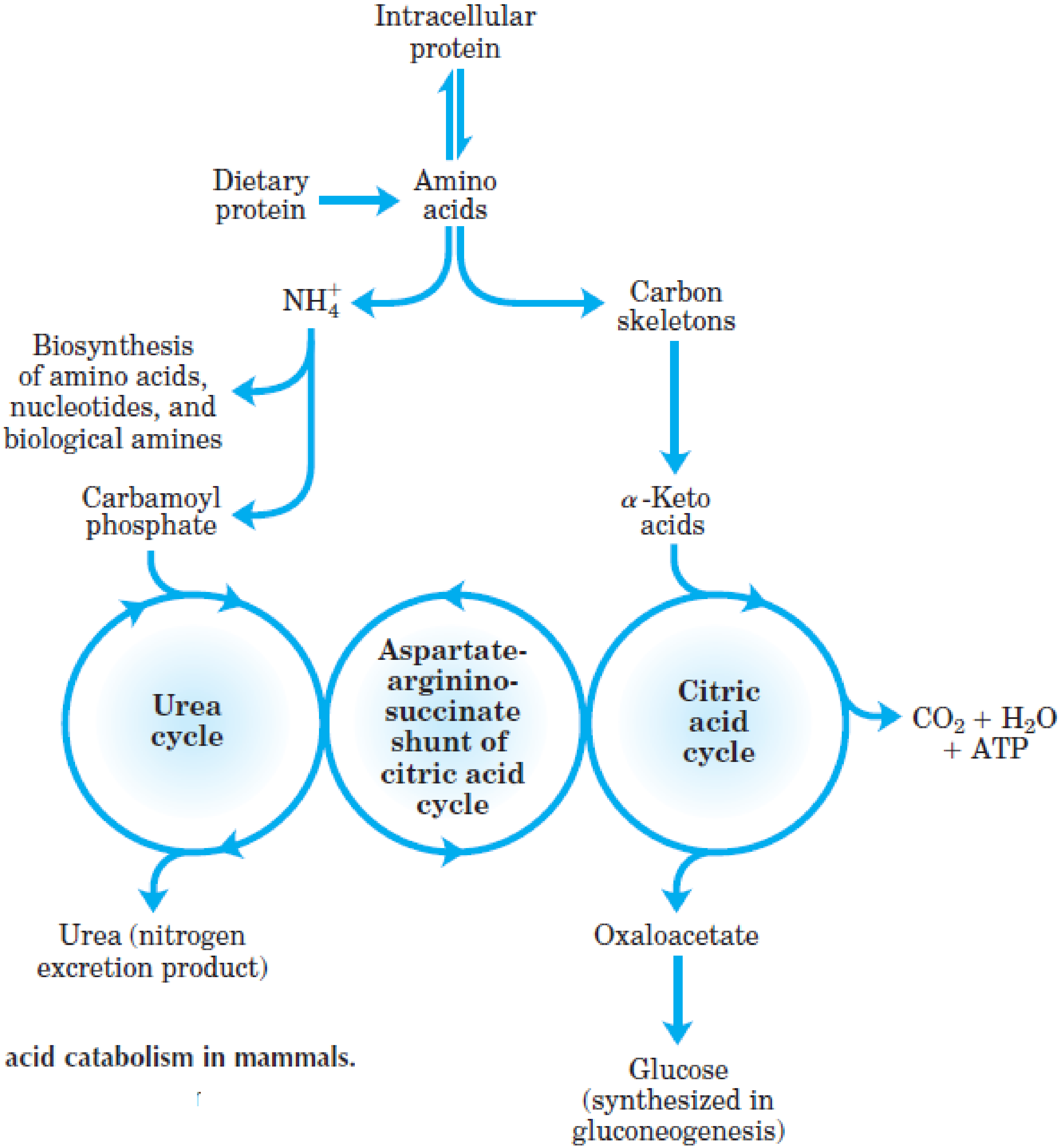


ЦТК


pyruvate
acetyl-CoA
H2O
CoA
oxaloacetate
citrate
isocitrate
NAD+
NADH, H+, CO2
α-ketoglutarate
CoA
NAD+
NADH, H+, CO2
succinyl-CoA
GDP, Pi
GTP
ADP
ATP
succinate
FAD
FADH2
fumarate
H2O
malate
NAD+
NADH, H+
COO-
C =O
CH2
COO-
COO-
CH2
HO–C–COO-
CH2
COO-
COO-
CH2
H–C–COO-
HO–C–H
COO-
COO-
CH2
CH2
C =O
COO-
COO-
CH2
CH2
C =O
S-CoA
COO-
CH2
CH2
COO-
H
C
COO-
C
H
-OOC
COO-
HO–C–H
CH2
COO-

Связь АК-катаболизма с ЦТК





Intracellular protein

Dietary protein

Amino acids

NH_4^+

Carbon skeletons

Biosynthesis of amino acids, nucleotides, and biological amines

Carbamoyl phosphate

α-Keto acids

Urea cycle

Aspartate-argininosuccinate shunt of citric acid cycle

Citric acid cycle

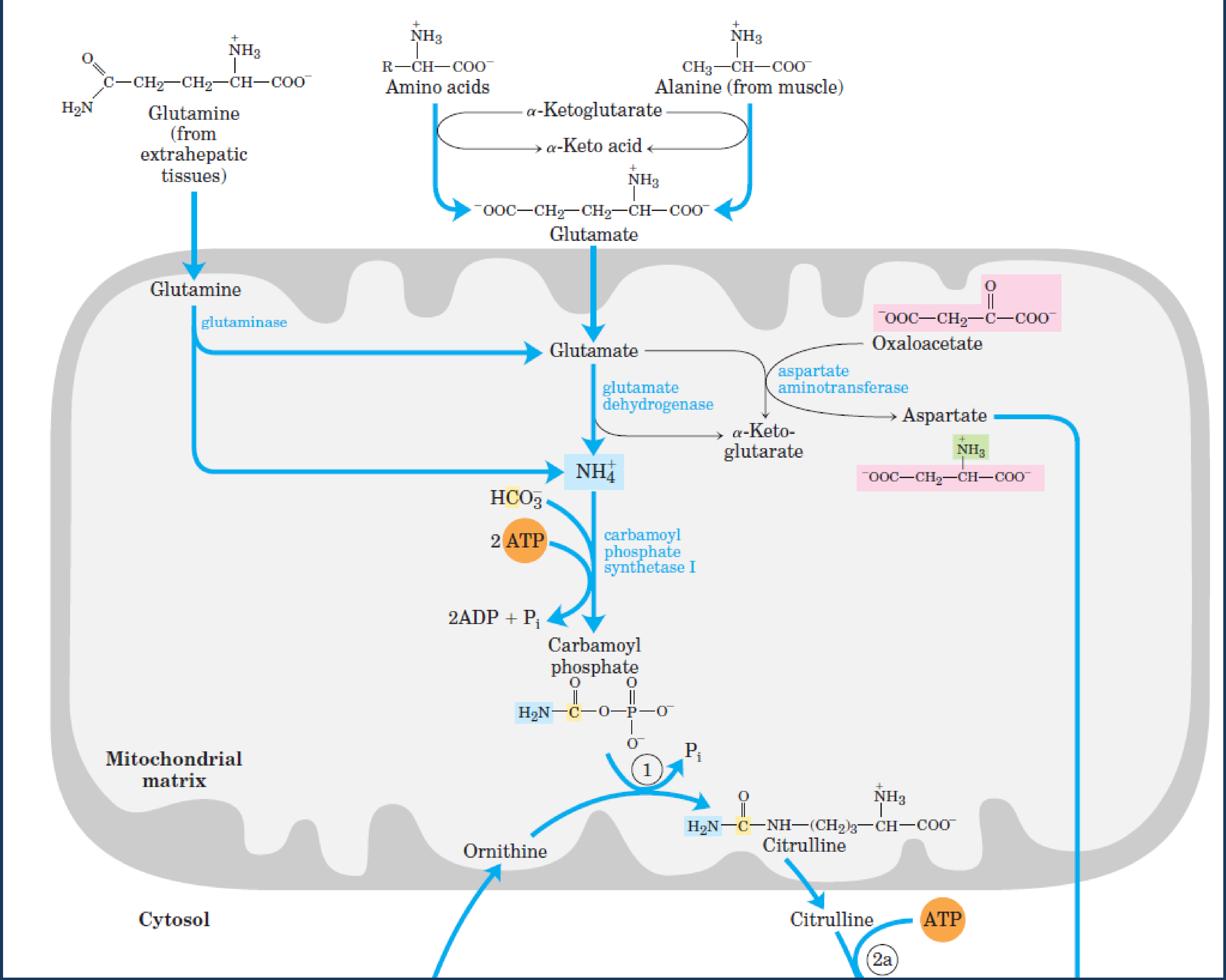
$CO_2 + H_2O$ + ATP

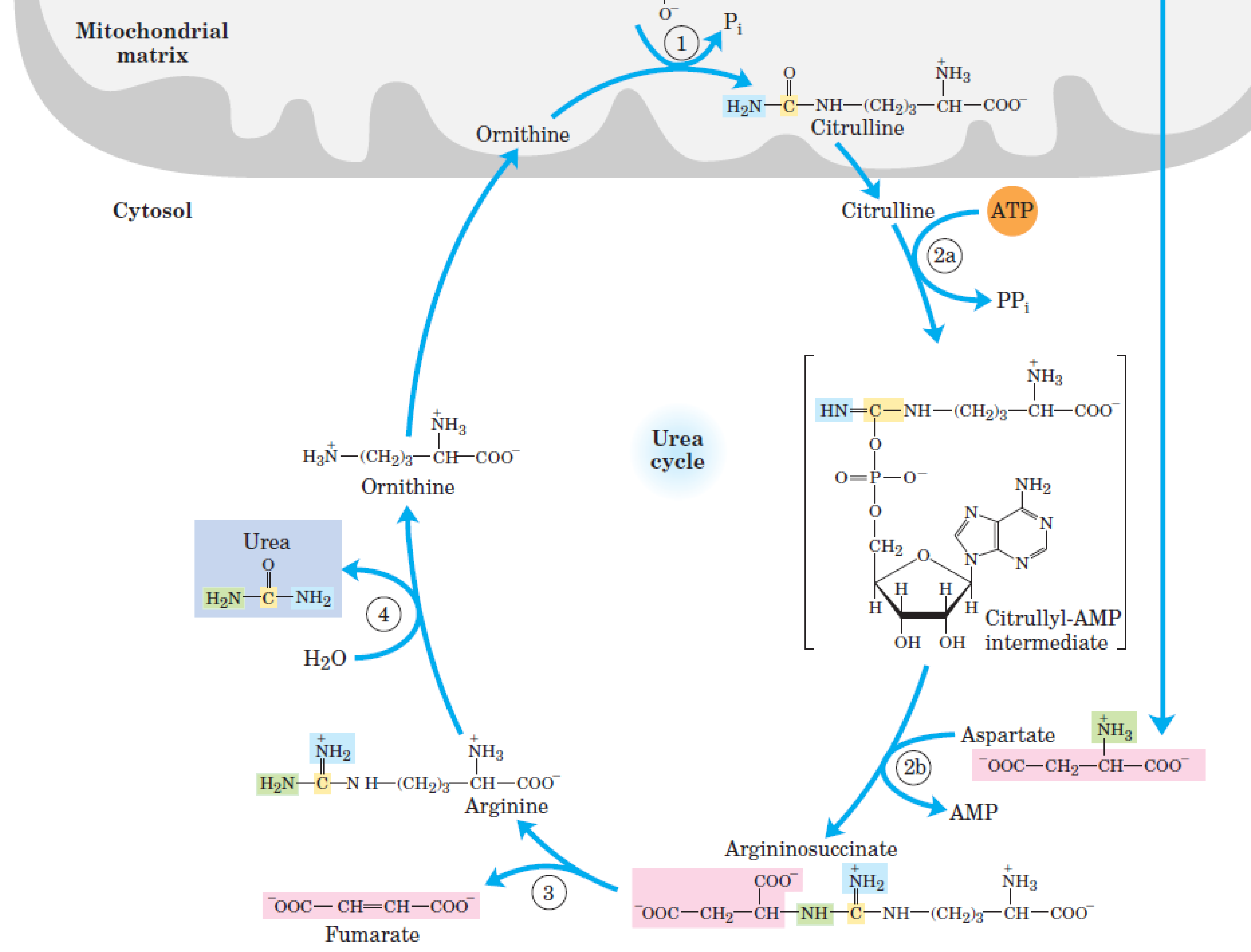
Urea (nitrogen excretion product)

Oxaloacetate

Glucose (synthesized in gluconeogenesis)

Overview of amino acid catabolism in mammals.


Glutamine (from extrahepatic tissues)
Amino acids
Alanine (from muscle)
α-Ketoglutarate
α-Keto acid
Glutamate
Glutamine
glutaminase
Glutamate
Oxaloacetate
aspartate aminotransferase
Aspartate
glutamate dehydrogenase
α-Ketoglutarate
NH$_4^+$
HCO$_3^-$
2 ATP
carbamoyl phosphate synthetase I
2ADP + P$_i$
Carbamoyl phosphate
P$_i$
1
Citrulline
Ornithine
Mitochondrial matrix
Cytosol
Citrulline
ATP
2a


Mitochondrial matrix
Cytosol
O⁻
①
Pᵢ
Ornithine
H₂N—C(=O)—NH—(CH₂)₃—CH(NH₃⁺)—COO⁻
Citrulline
Citrulline
ATP
2a
PPᵢ
HN=C(—O—P(=O)(O⁻)—O—CH₂-ribose-adenine)—NH—(CH₂)₃—CH(NH₃⁺)—COO⁻
Citrullyl-AMP intermediate
Urea cycle
Aspartate
⁻OOC—CH₂—CH(NH₃⁺)—COO⁻
2b
AMP
Argininosuccinate
⁻OOC—CH₂—CH(COO⁻)—NH—C(=NH₂⁺)—NH—(CH₂)₃—CH(NH₃⁺)—COO⁻
3
⁻OOC—CH=CH—COO⁻
Fumarate
H₂N—C(=NH₂⁺)—NH—(CH₂)₃—CH(NH₃⁺)—COO⁻
Arginine
4
H₂O
Urea
H₂N—C(=O)—NH₂
H₃N⁺—(CH₂)₃—CH(NH₃⁺)—COO⁻
Ornithine

Окислительные реакции в пероксисомах

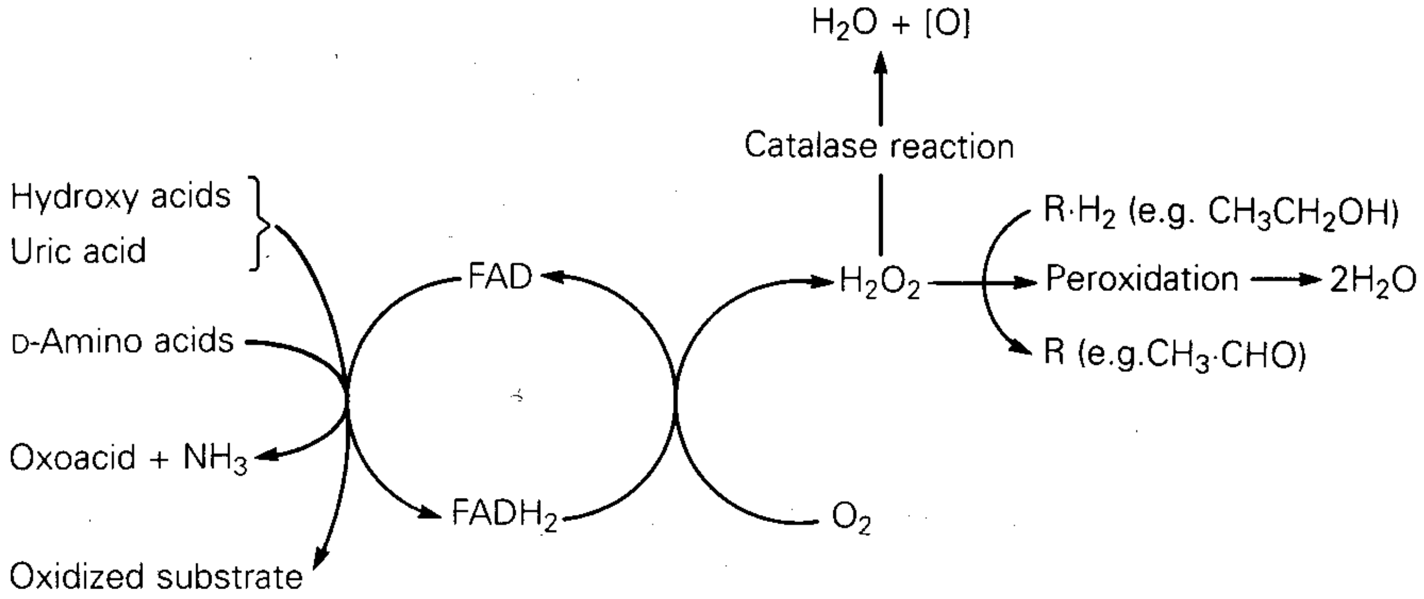


Fig. 1.64 Oxidative reactions of peroxisomes

Metabolic Functions of Peroxisomes

Mammals

Biosynthesis: ether phospholipids (plasmalogens), cholesterol and bile acids, polyunsaturated fatty acids

Degradation: amino acids, purines, **prostaglandin, polyamines**, α-oxidation of fatty acids, β-oxidation of fatty acids, decomposition of hydrogen peroxide

Humans

Biosynthesis: ether phospholipids (plasmalogens), cholesterol and bile acids, polyunsaturated fatty acids

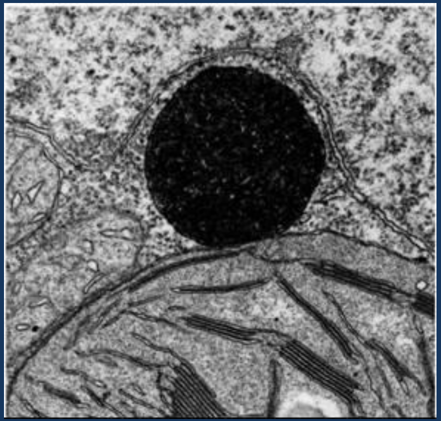
Degradation: amino acids, purines, α-oxidation of fatty acids, β-oxidation of fatty acids, decomposition of hydrogen peroxide

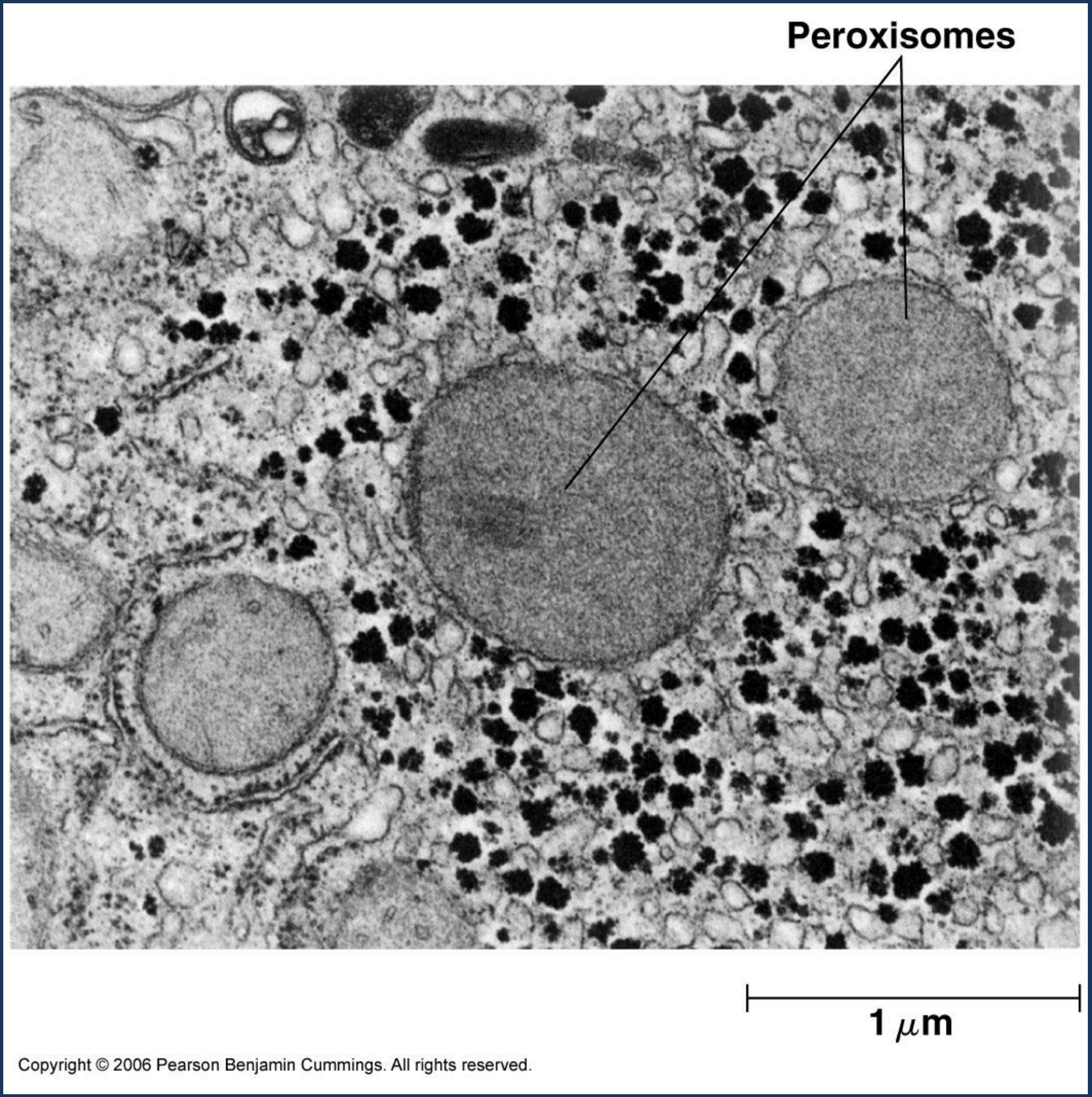
Animal Peroxisomes

Single membrane

Roughly spherical
0.2 - 1.7μm

Composition
varies





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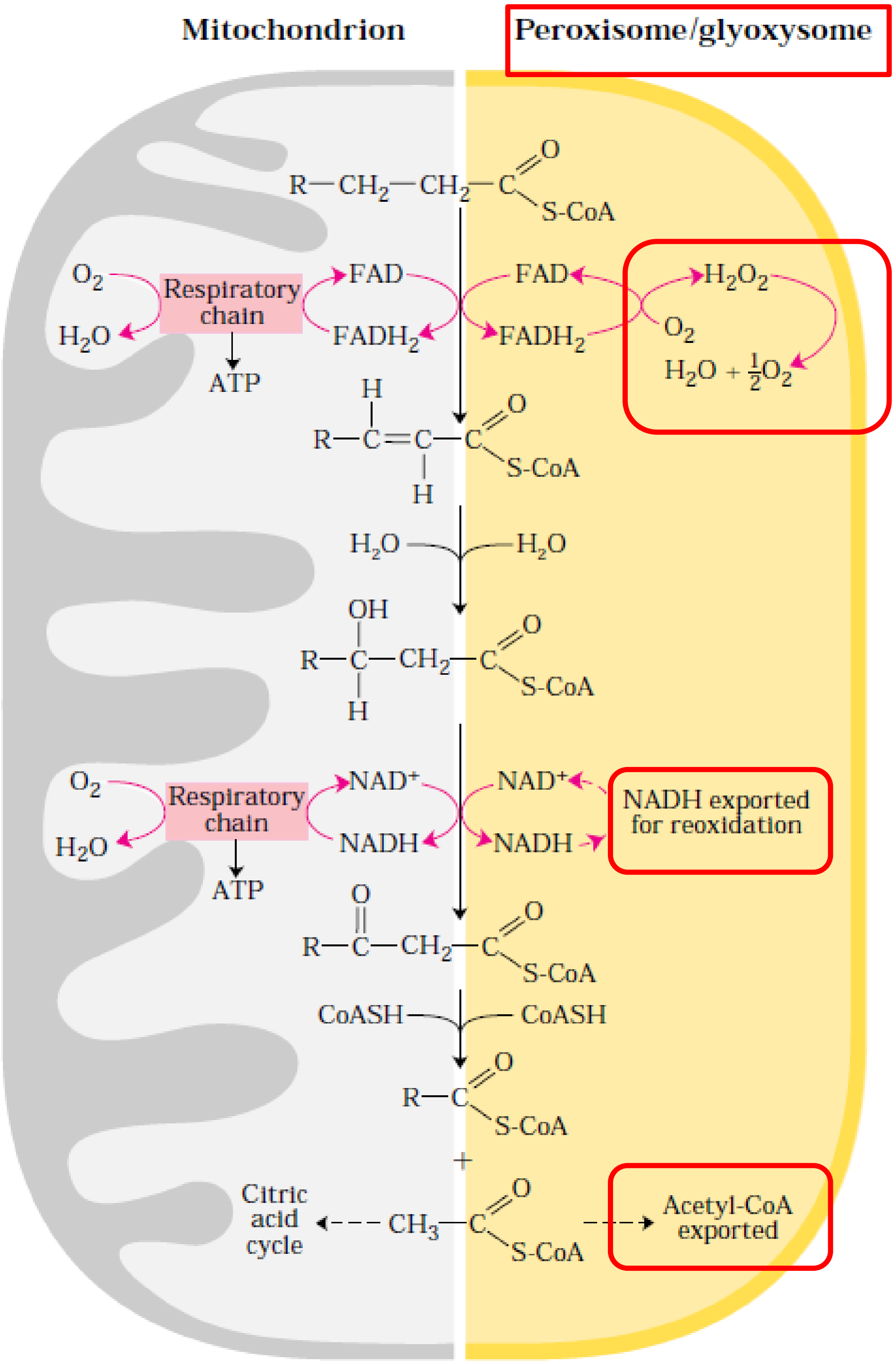
Metabolic Functions of Peroxisomes

Yeasts
Biosynthesis: lysine
Degradation: amino acids, methanol, β-oxidation of fatty acids, decomposition of hydrogen peroxide, **glyoxylate cycle**

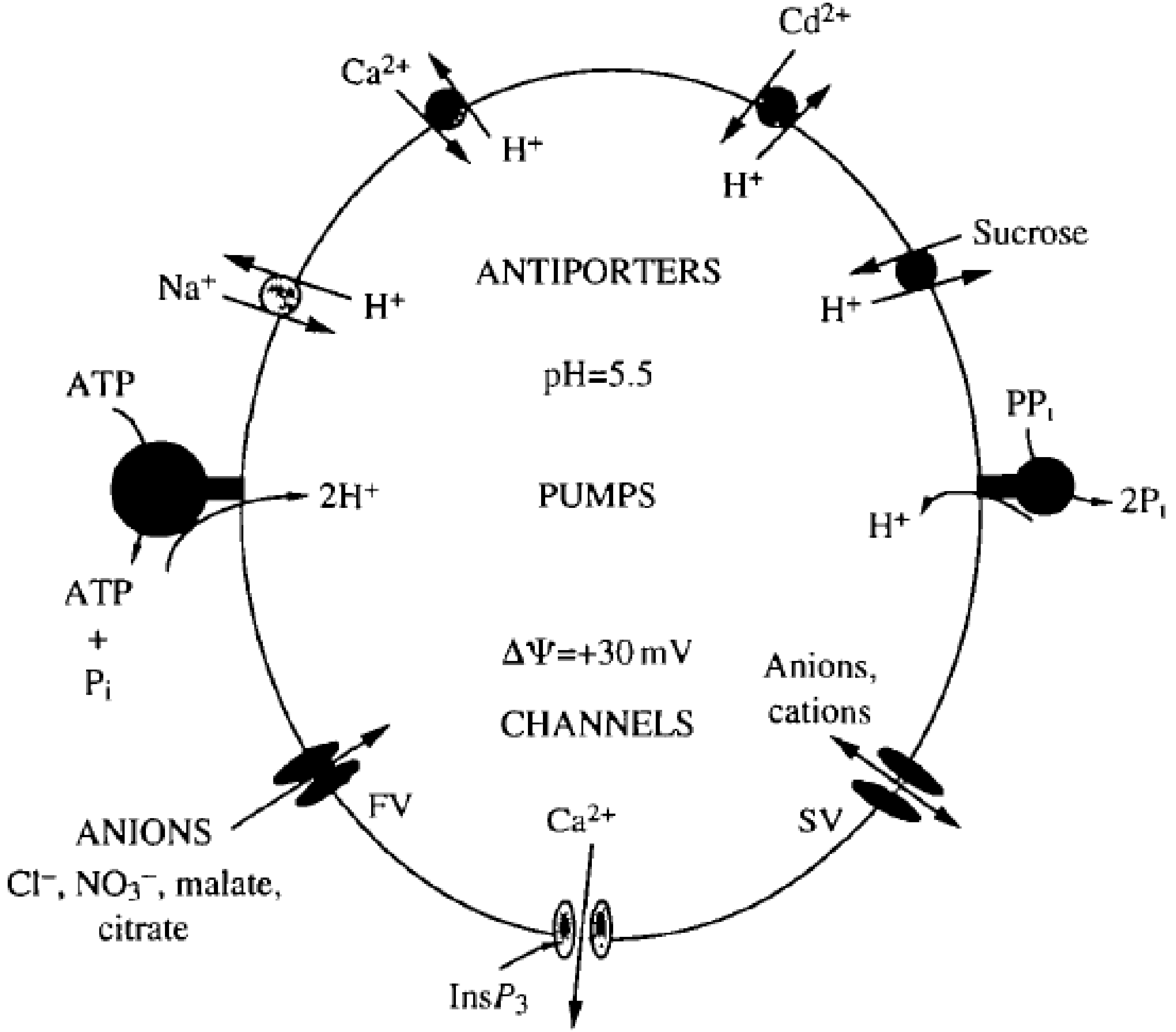
Fungi
Biosynthesis: penicillin
Degradation: β -oxidation of fatty acids, decomposition of hydrogen peroxide, **glyoxylate cycle**

Plants
Degradation: purines, some reactions of **photorespiration** (the conversion of glycolate to glycine and of serine to glycerate), β-oxidation of fatty acids, decomposition of hydrogen peroxide, **glyoxylate cycle**

β -oxidation of fatty acids



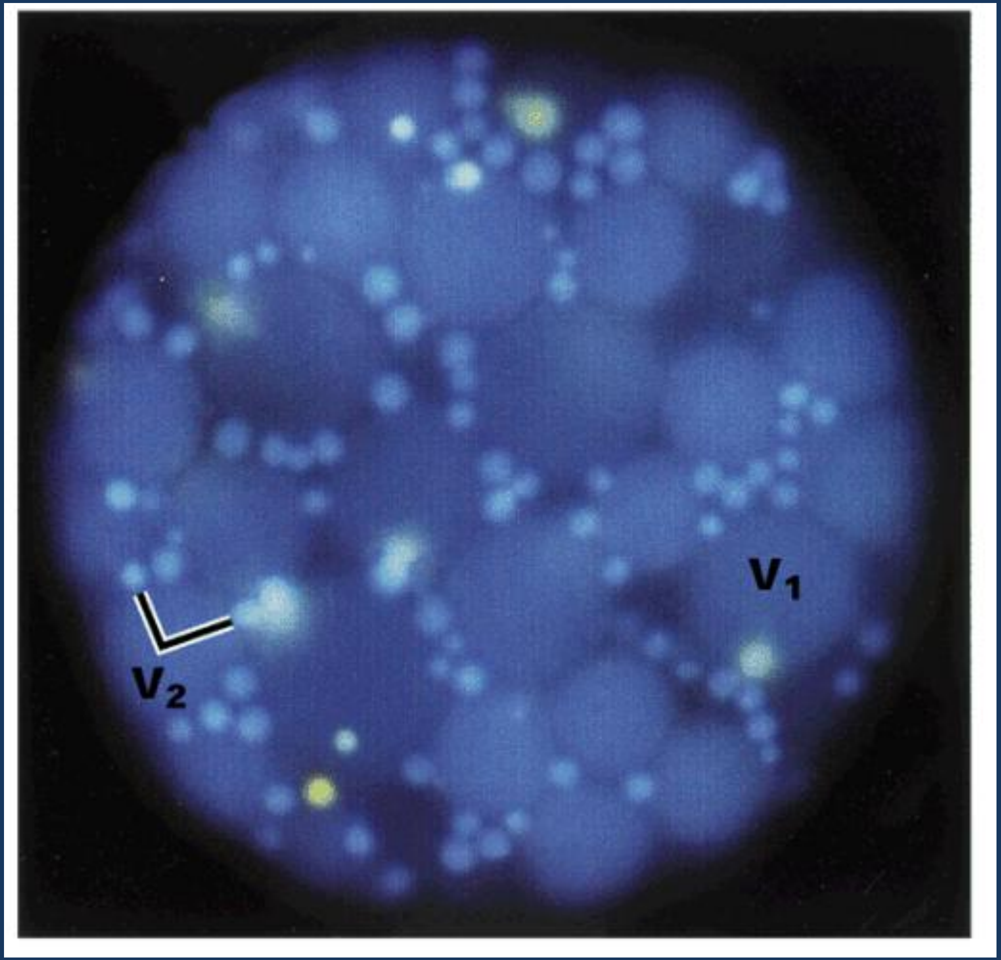
The plant vacuole



Plant vacuoles: metabolic roles

- (1) Storage: sugars,polysaccharides, organic acids and proteins. Primary metabolites can be retrieved from the vacuole and utilized in metabolic pathways.
- (2) Toxic avoidance. By accumulating heavy metals, such as Cd and Na,the vacuole can be viewed as a micro-kidney inside each plant cell, filtering and sequestering potentially toxic ions from the cytosol.
- (3) pH and ionic homeostasis. Reactions in the cytosol are exquisitely sensitive to changes in pH and ionic strength. The pH of the vacuole of higher plants is typically 5.0-5.5, but can reach as low as 2.5 in lemon fruits. The most acidic vacuole belongs to the brown alga Desmerestia, with a lumenal pH of 0.6 due to the accumulation of H2SO4.

- (4) *Defense against microbial pathogens* - large quantities of bitter-tasting phenolic compounds, cyanogenic glycosides or alkaloids accumulate in vacuoles. Chitinase is specifically synthesized in response to wounding and accumulates in the vacuoles of bean plants. Secondary toxic products for self defense purposes: nicotine, caffeine, taxol, opium, …
- (5) *Pigmentation. Many plant cells accumulate water-soluble flavonoid pigments* called anthocyanins, which range in color from orange-red to purple. In leaf tissue, such pigments are concentrated in the vacuoles of epidermal cells, where they probably prevent photooxidation of the photosynthetic apparatus by lowering the light intensity and ultraviolet irradiation.
- (6) *Lysosomes. Like the lysosomes of animal cells, plant vacuoles contain a variety of* acid hydrolases, such as proteases, ribonucleases and glycosidases



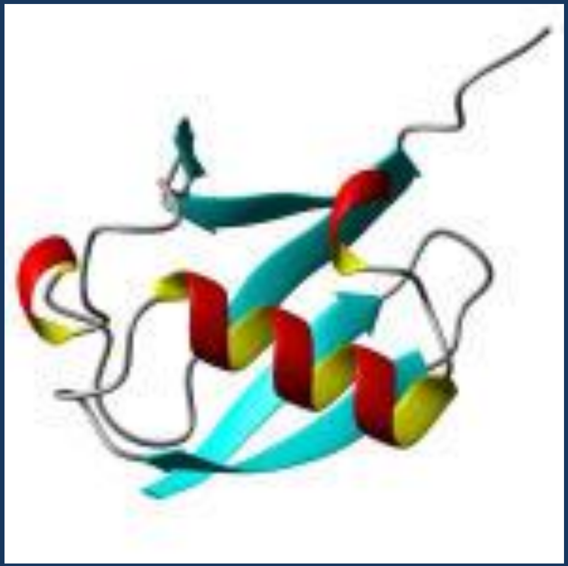
The bigger vacuoles are **protein storage** and
the small ones are
lytic vacuoles—in single type of cells (aleurone cell from a barley seed).

Виды катаболических процессов в цитозоле

- Расщепление полимеров до мономерных звеньев (полисахаридов, полипептидов, НК)
- Катаболизм моносахаридов (гликолиз)
- Катаболизм аминокислот

- --
- cDNA digestion by cytosolic nucleases occur when the decomplexed transgene is present in the cytosol/ Ca2+-sensitive cytosolic nuclease.

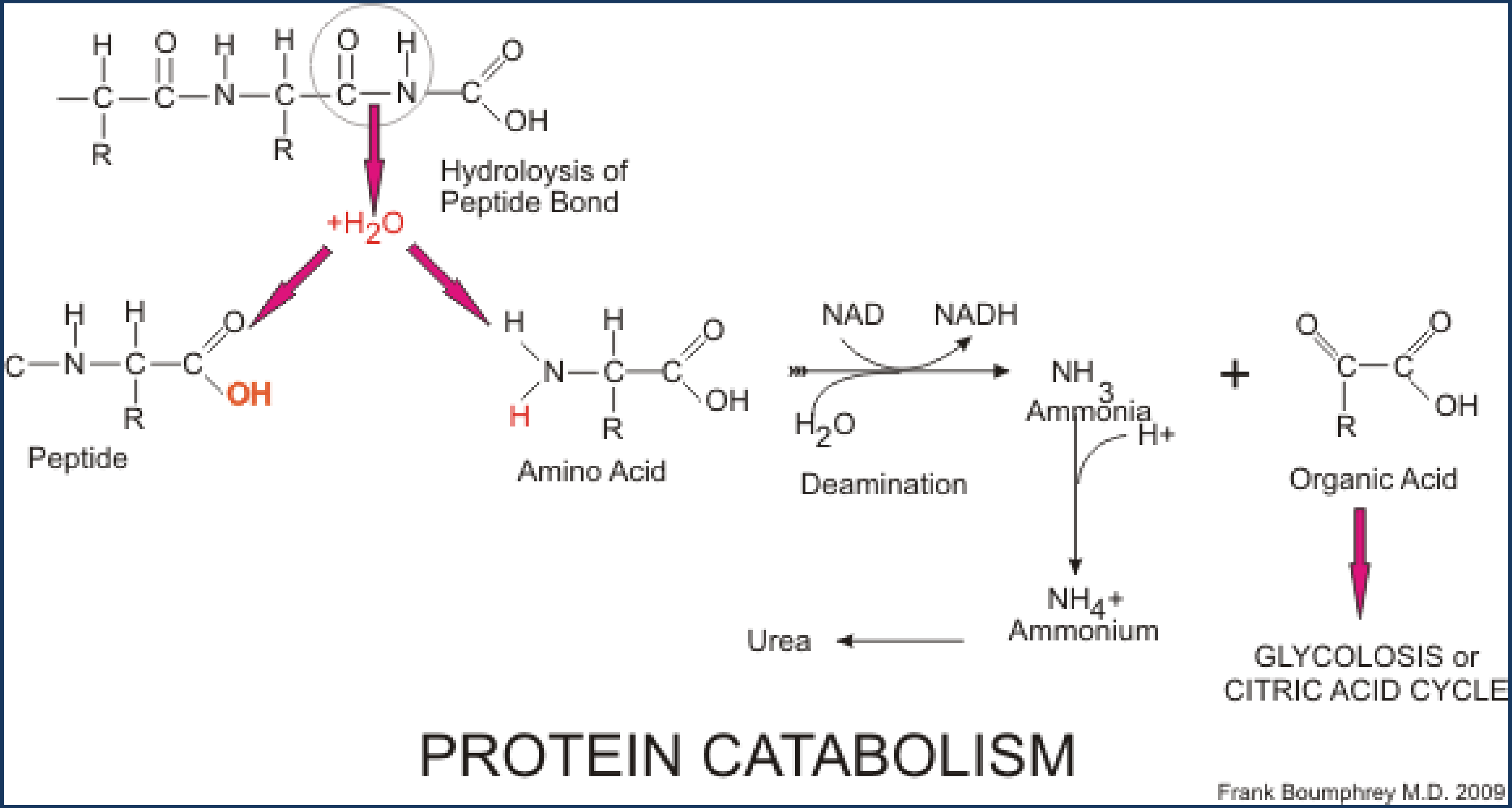
Proteolysis in cytosol



UBIQUITIN

- UBIQUITINATION

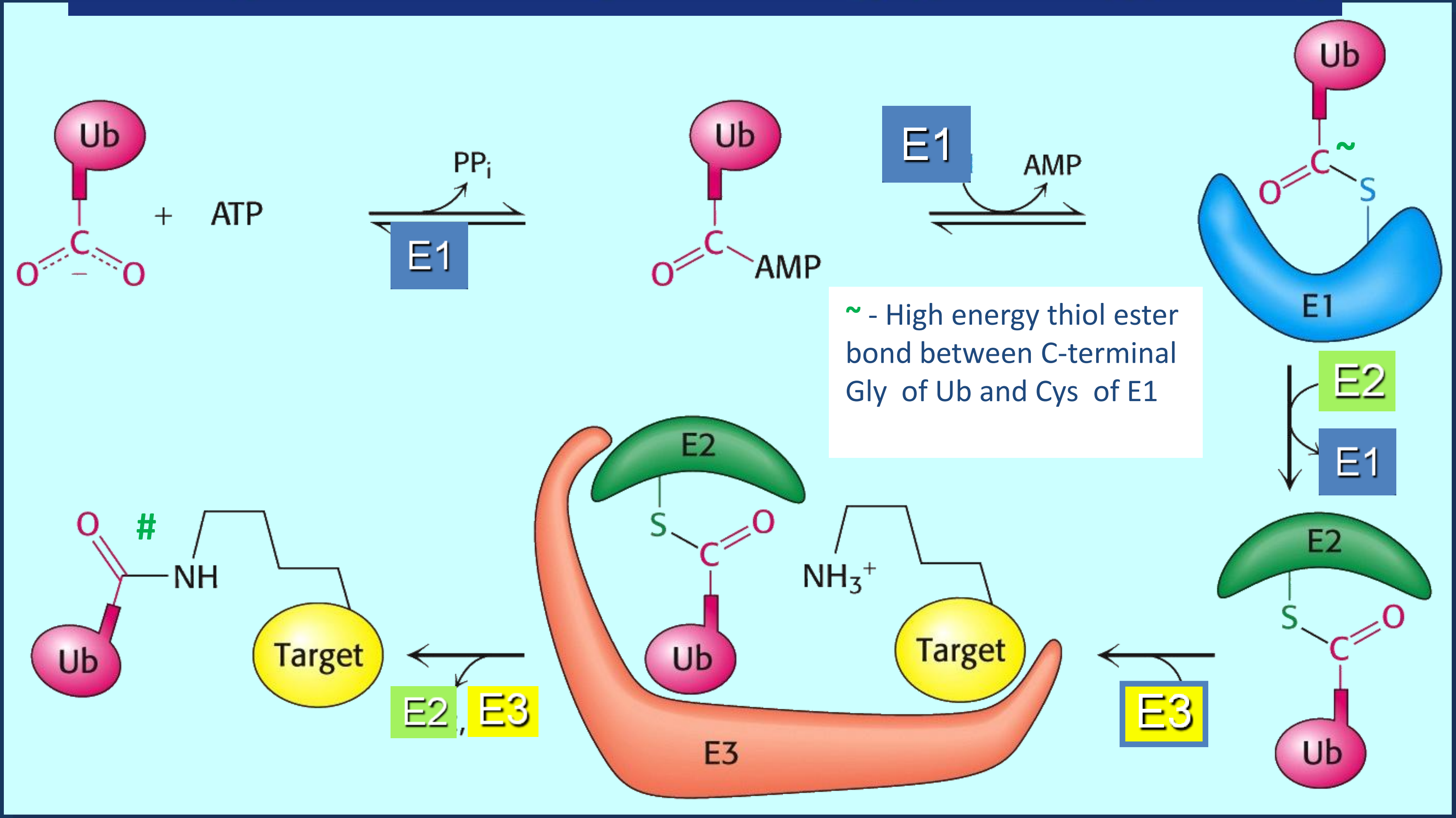
In general, multiple ubiquitin units are arranged in polyubiquitin chains linked via Lys_{48} of ubiquitin, targeting the protein for degradation



PROTEIN CATABOLISM

Frank Boumphrey M.D. 2009

Ubiquitination pathway (3 enzymes)



~ - High energy thiol ester bond between C-terminal Gly of Ub and Cys of E1

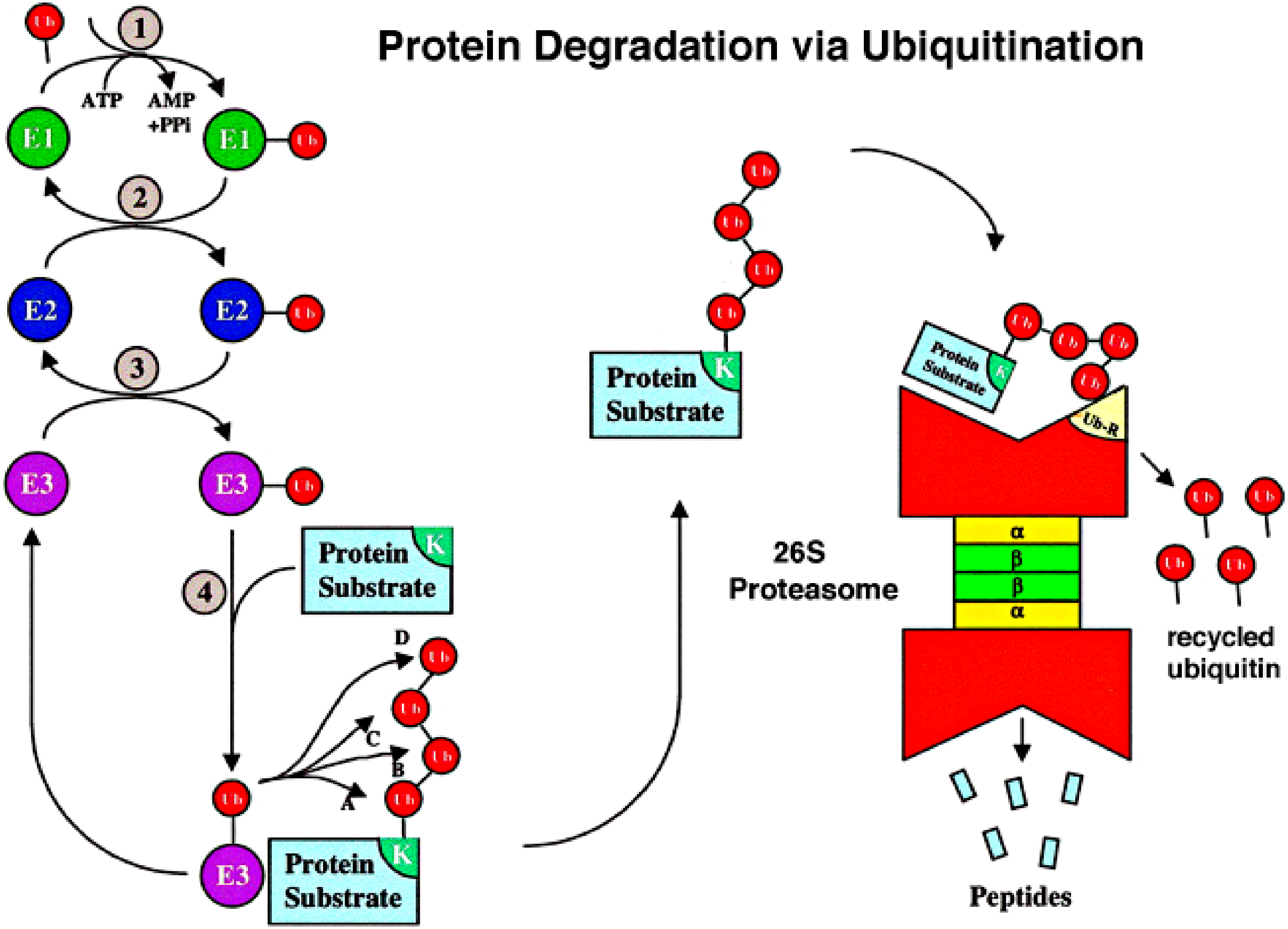
E1 Ubiquitin (Ub) activating enzyme E_1

E2 Ubiquitin conjugating enzymes E_2

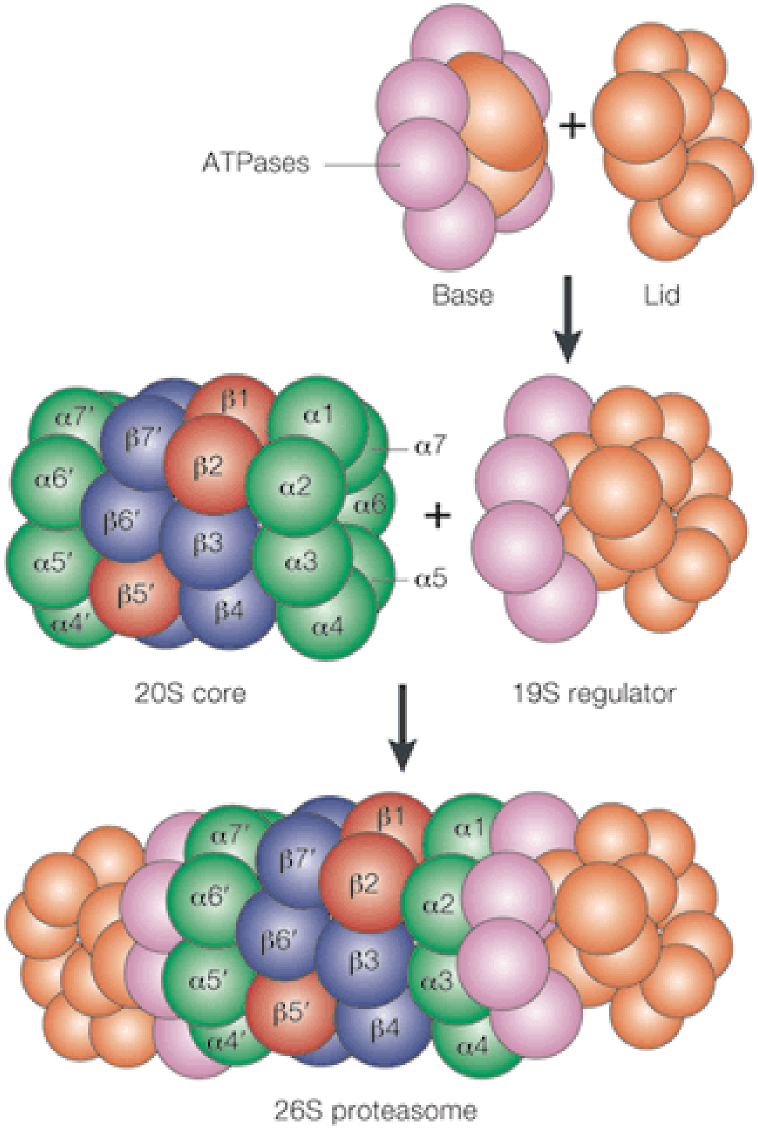
E3 Ubiquitin ligase E_3

# - isopeptide bond between C-terminal Gly of Ub and ε-NH_2 of Lys on a target protein

Protein Degradation via Ubiquitination



THE PROTEASOME



Nature Reviews | Molecular Cell Biology

Mono-Ub: Another Function

- Histone regulation
- Endocytosis
- Budding of retroviruses from plasma membrane

Биотрансформация

с/к «Биохимия клетки»
Лекция 2-4

Drug Metabolism - Phase I

- Phase I Reactions
  - Reduction
  - Hydrolytic cleavage
  - Oxidation
  - Alkylation (Methylation)
  - Dealkylation
  - Ring cyclization
  - N-carboxylation
  - Dimerization
  - Transamidation
  - Isomerization
  - Decarboxylation

Drug Metabolism - Oxidation

Two types of oxidation reactions:

- Oxygen is incorporated into the drug molecule (e.g. hydroxylation)
- Oxidation causes the loss of part of the drug molecule

  (e.g. oxidative deimination, dealkylation)

Microsomal Mixed Function Oxidases (MFOs)

- "Microsomes"

  form in vitro after cell homogenization and fractionation of ER
  - Rough microsomes are primarily associated with protein synthesis
  - Smooth microsomes contain a class of oxidative enzymes called
- "Mixed Function Oxidases" or "Monooxygenases"
  - These enzymes require a reducing agent (NADPH) and molecular oxygen (one oxygen atom appearing in the product and the other in the form of water)

Drug Metabolism - Oxidation

- MFO consists of two enzymes:
  - Flavoprotein, NADPH-cytochrome c reductase
    - One mole of this enzyme contains one mole each of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD)
    - Enzyme is also called NADPH-cytochrome P450 reductase
  - Cytochrome P450
    - named based on its light absorption at 450 nm when complexed with carbon monoxide
    - is a hemoprotein containing an iron atom which can alternate between the ferrous (Fe^{++}) and ferric (Fe^{+++}) states
    - Electron acceptor
    - Serves as terminal oxidase
    - its relative abundance compared to NADPH-cytochrome P450 reductase makes it the rate-limiting step in the oxidation reactions

Drug oxidation requires:

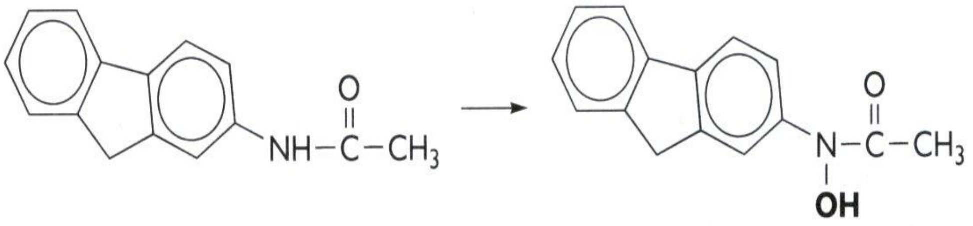
Cytochrome P450
Cytochrome P450 reductase
NADPH
Molecular oxygen

The cycle involves four steps:

1. Oxidized (Fe3+) cytochrome P-450 combines with a drug substrate to form a binary complex.
2. NADPH donates an electron to the cytochrome P-450 reductase, which in turn reduces the oxidized cytochrome P-450-drug complex.
3. A second electron is introduced from NADPH *via* the same cytochrome P-450 reductase, which serves to reduce molecular oxygen and form an "activated oxygen"-cytochrome P-450-substrate complex.
4. This complex in turn transfers "activated" oxygen to the drug substrate to form the oxidized product. The potent oxidizing properties of this activated oxygen permit oxidation of a large number of substrates.

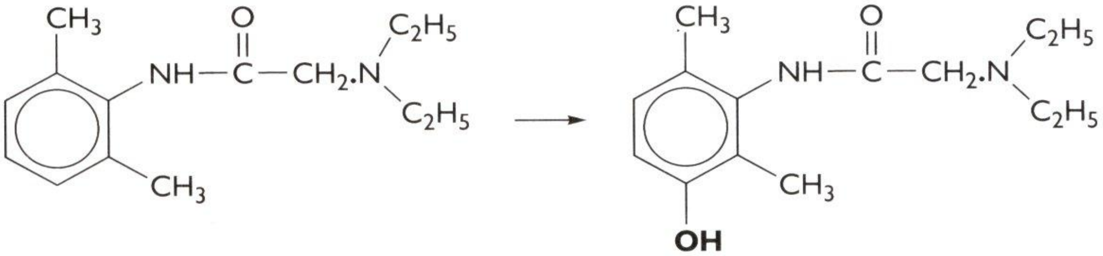
Реакции окисления с молекулярным кислородом

Aromatic hydroxylation:



The N-hydroxylation of 2-acetylaminofluorene.

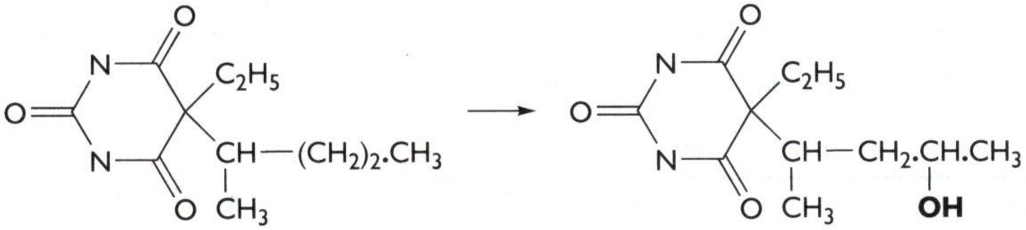
N-гидроксилирование



The 3-hydroxylation of lignocaine.

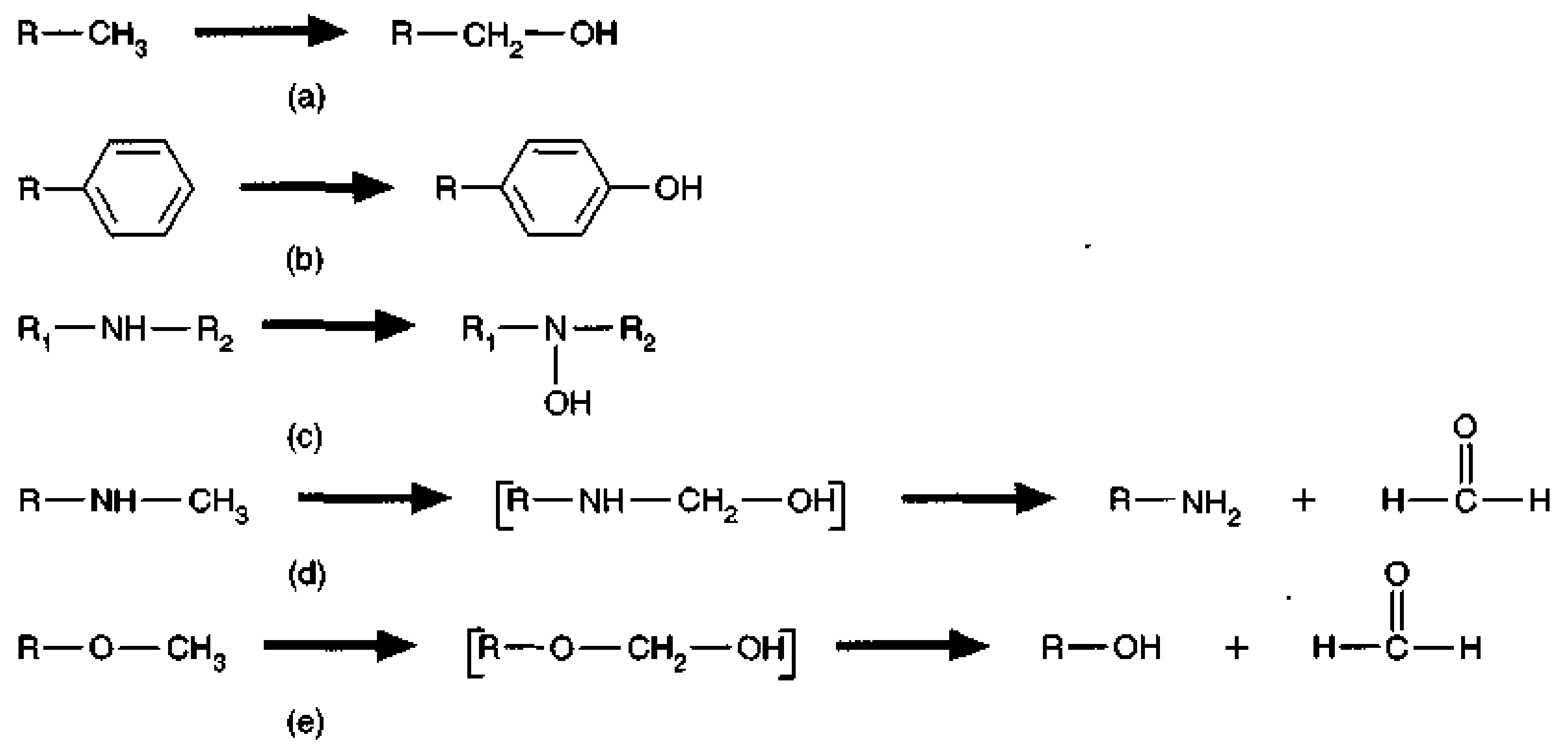
C- гидроксилирование

Aliphatic hydroxylation:C

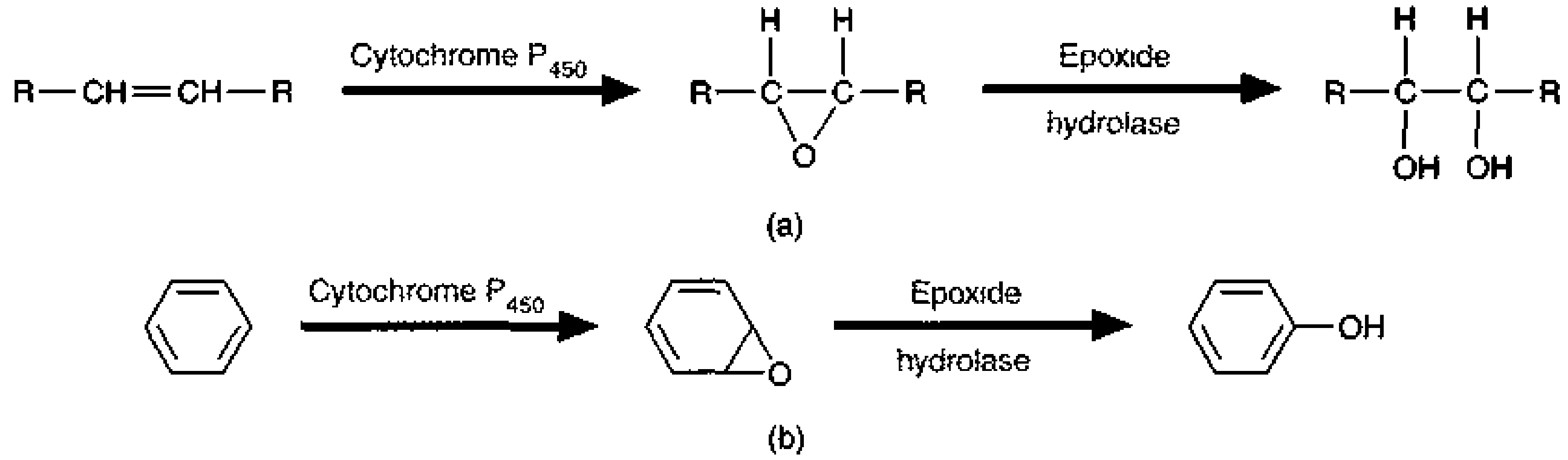


The side-chain hydroxylation of pentobarbitone.

Разнообразие реакций гидроксилирования, катализируемых цитохромами Р450: образование спиртов


R—CH3 → R—CH2—OH
(a)
R → R OH
(b)
R1—NH—R2 → R1—N—R2 OH
(c)
R—NH—CH3 → [R—NH—CH2—OH] → R—NH2 + H—C—H O
(d)
R—O—CH3 → [R—O—CH2—OH] → R—OH + H—C—H O
(e)

Реакции образования и превращения эпоксидов, катализируемые цитохромами P450



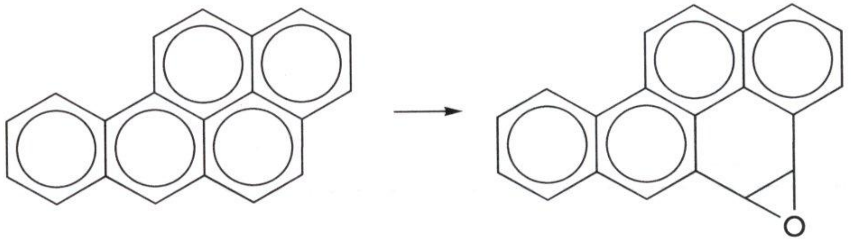
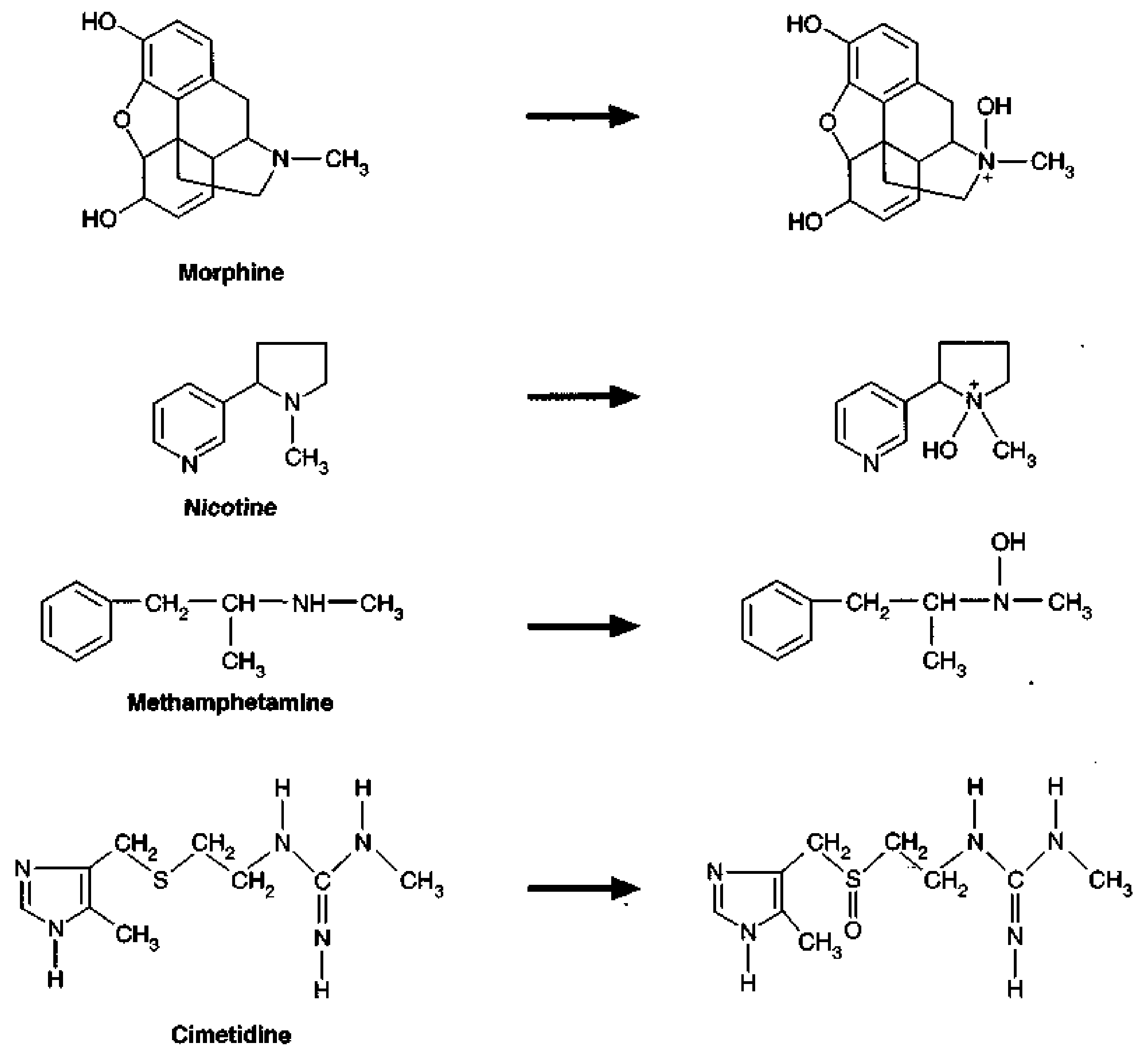
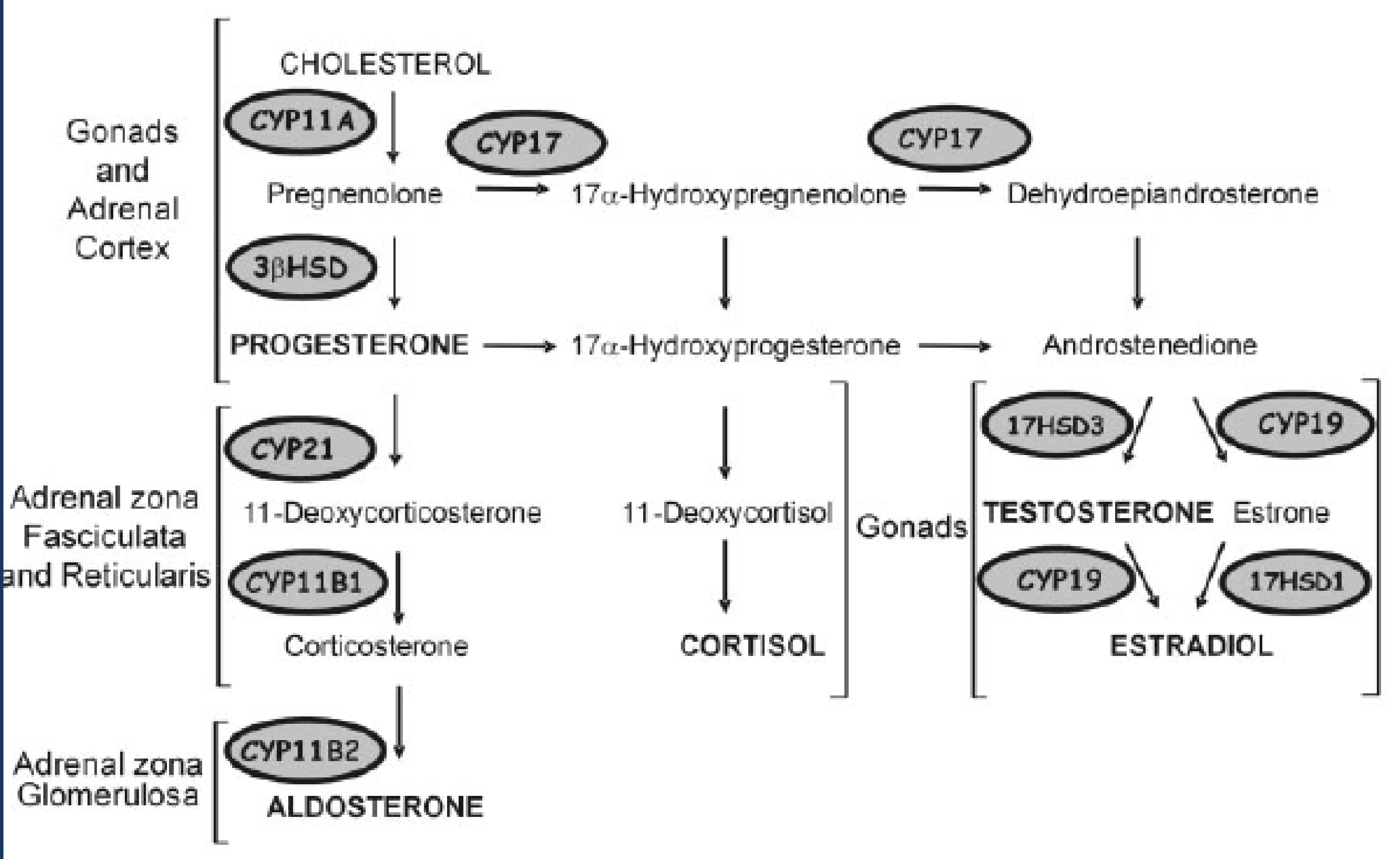


Figure 1.3 The formation of benzo[*a*]pyrene-4,5-epoxide.

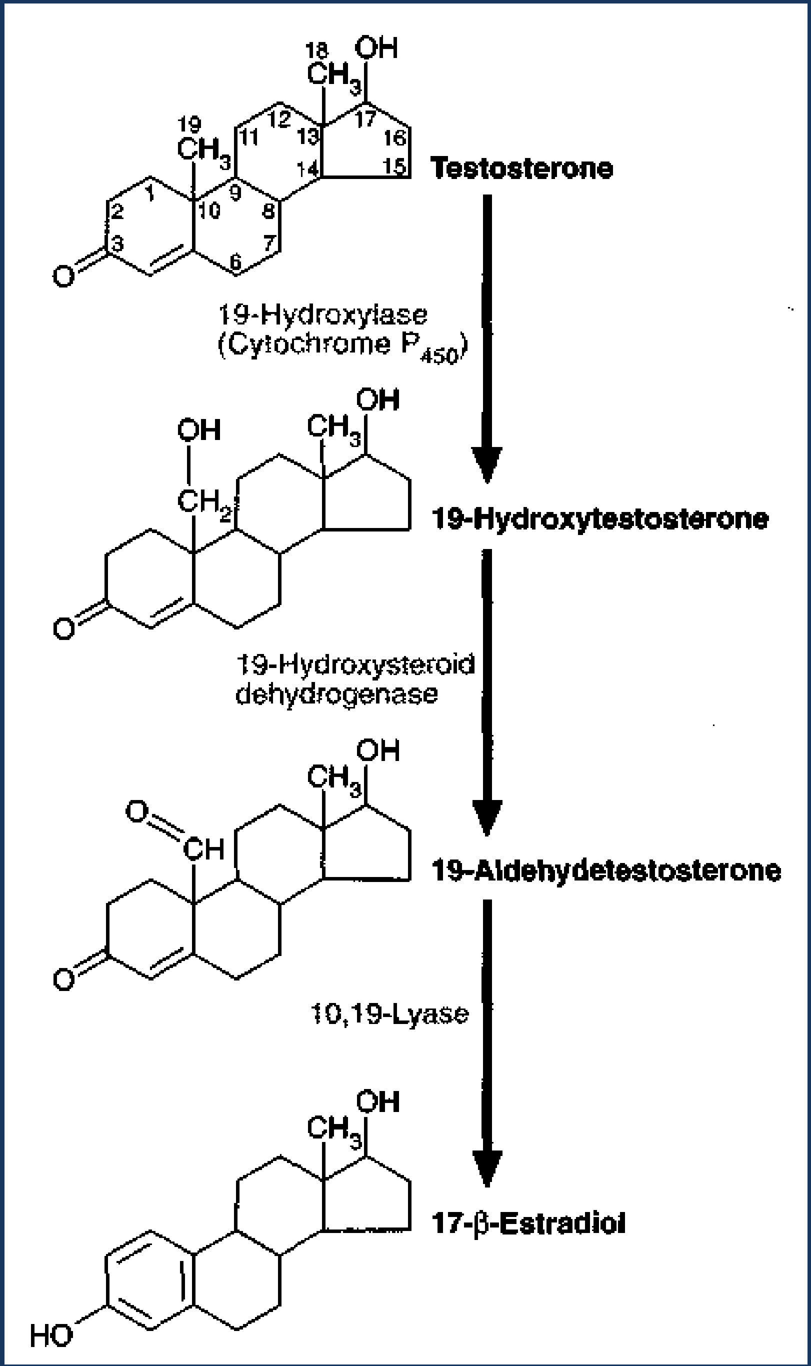


Примеры реакций цитохромов P450 с субстра-тами

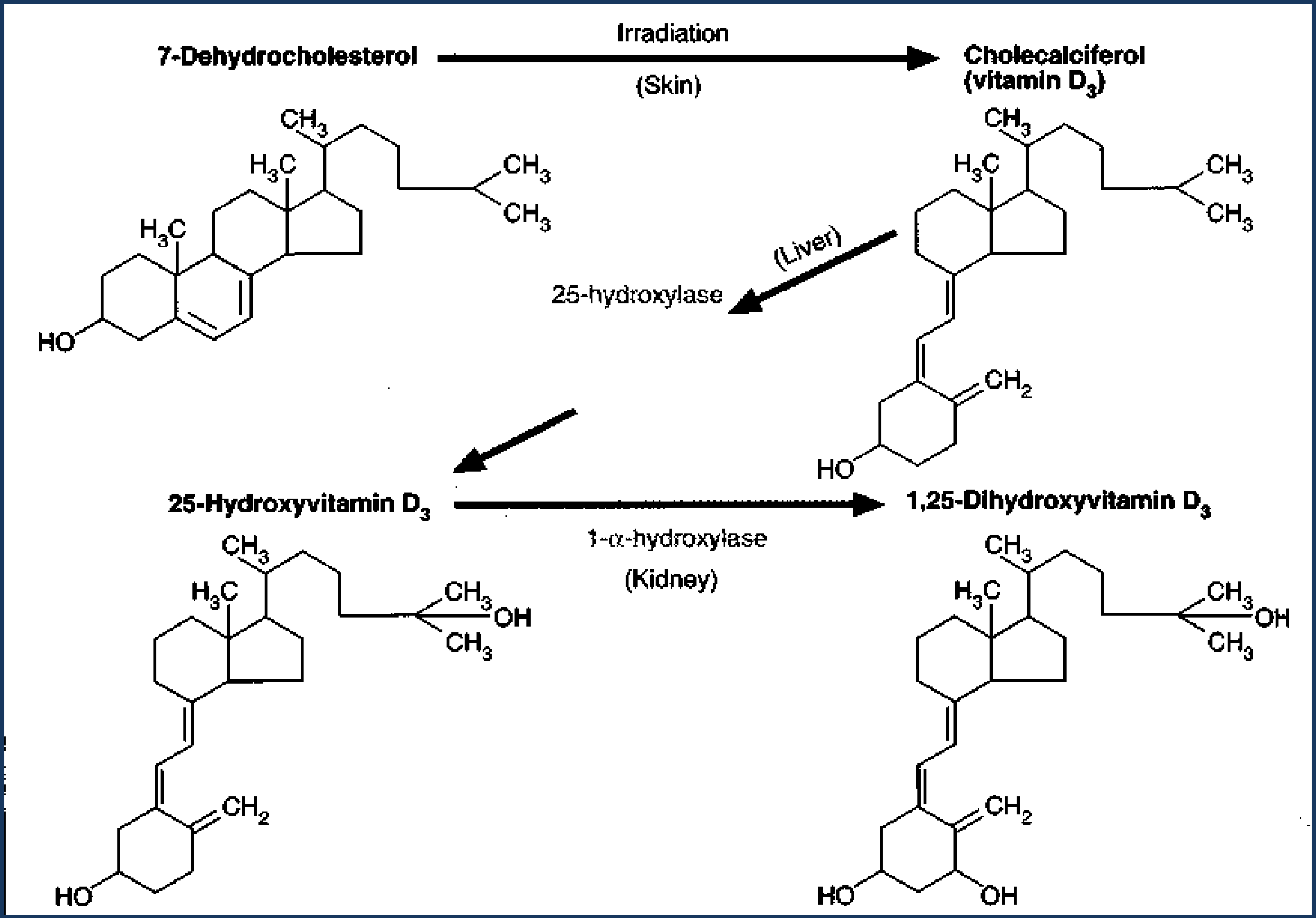
Пути синтеза стероидных гормонов в железах внутренней секреции



Работа ароматазной системы



Реакции гидроксилирования в синтезе витамина Д (цитохромы Р450)



- **CYP1 drug metabolism (3 subfamilies, 3 genes, 1 pseudogene)**
- **CYP2 drug and steroid metabolism (13 subfamilies, 16 genes, 16 pseudogenes)**
- **CYP3 drug metabolism (1 subfamily, 4 genes, 2 pseudogenes)**
- **CYP4 arachidonic acid or fatty acid metabolism (5 subfamilies, 11 genes, 10**
- **pseudogenes)**
- **CYP5 Thromboxane A2 synthase (1 subfamily, 1 gene)**
- **CYP7A bile acid biosynthesis 7-alpha hydroxylase of steroid nucleus (1**
- **subfamily member)**
- **CYP7B brain specific form of 7-alpha hydroxylase (1 subfamily member)**
- **CYP8A prostacyclin synthase (1 subfamily member)**
- **CYP8B bile acid biosynthesis (1 subfamily member)**
- **CYP11 steroid biosynthesis (2 subfamilies, 3 genes)**
- **CYP17 steroid biosynthesis (1 subfamily, 1 gene) 17-alpha hydroxylase**
- **CYP19 steroid biosynthesis (1 subfamily, 1 gene) aromatase forms estrogen**
- **CYP20 Unknown function (1 subfamily, 1 gene)**
- **CYP21 steroid biosynthesis (1 subfamily, 1 gene, 1 pseudogene)**
- **CYP24 vitamin D degradation (1 subfamily, 1 gene)**
- **CYP26A retinoic acid hydroxylase important in development (1 subfamily member)**
- **CYP26B probable retinoic acid hydroxylase (1 subfamily member)**
- **CYP26C probabvle retinoic acid hydroxylase (1 subfamily member)**
- **CYP27A bile acid biosynthesis (1 subfamily member)**
- **CYP27B Vitamin D3 1-alpha hydroxylase activates vitamin D3 (1 subfamily member)**
- **CYP27C Unknown function (1 subfamily member)**
- **CYP39 unknown function (1 subfamily member)**
- **CYP46 cholesterol 24-hydroxylase (1 subfamily member)**
- **CYP51 cholesterol biosynthesis (1 subfamily, 1 gene, 3 pseudogenes) lanosterol**
- **14-alpha demethylase**

Сравнительная схема работы ЭТЦ системы цитохромов Р450 в митохондриях (А) и микросомах (В)

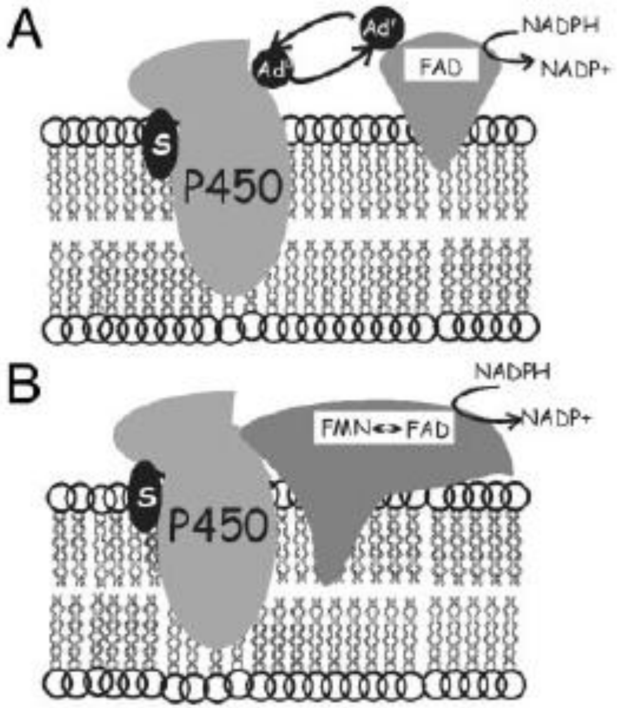
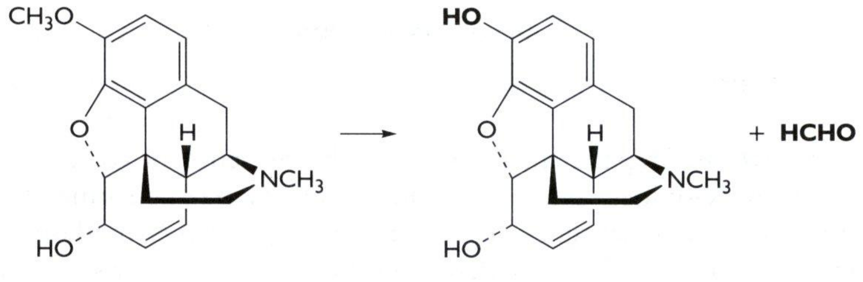


FIG. 2. Schematic representation of the mitochondrial electron transfer system (A) and microsomal electron transfer system (B). FAD, Flavin adenine dinucleotide; FMN, flavin mononucleotide; Ad^{r}, adrenodoxin reductase; Ad^{o}, adrenodoxin; S, substrate.

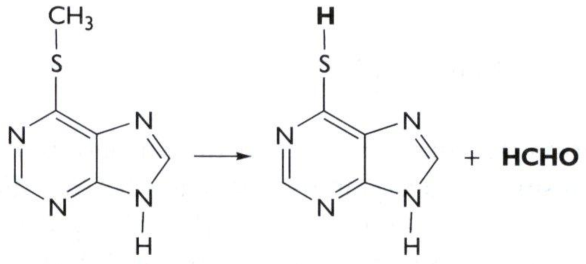
Drug Metabolism - Oxidation

O-demethylation:



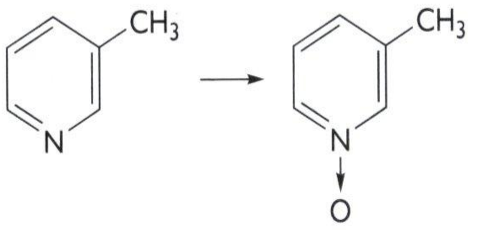
The *O*-demethylation of codeine.

S-demethylation:



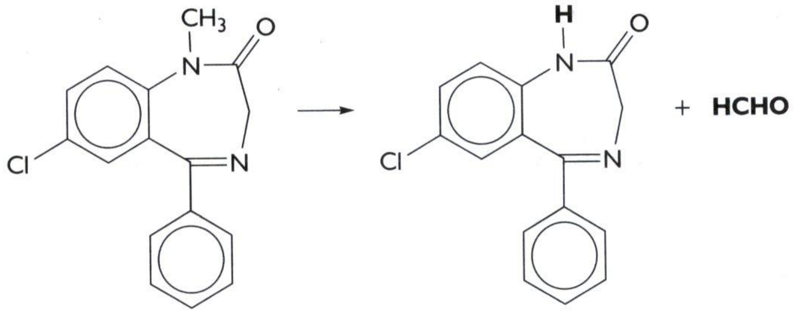
The *S*-demethylation of *S*-methylthiopurine.

N-oxidation:



The *N*-oxidation of 3-methylpyridine.

N-demethylation:



The *N*-demethylation of diazepam.

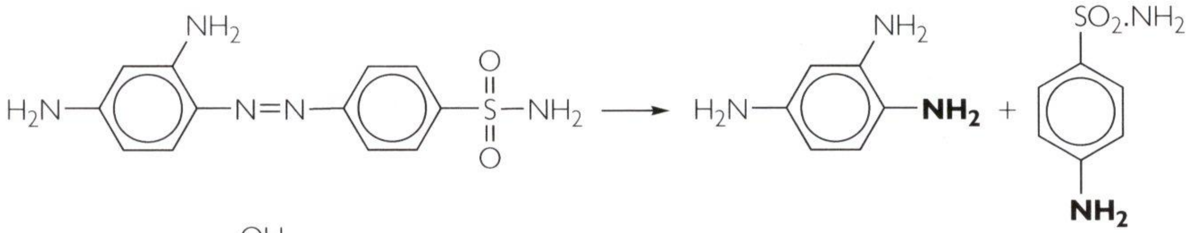
Monoamine Oxidases (MAO):

- Catalyze oxidative **deamination** of endogenous catecholamines (epinephrine)
- Located in nerve terminals and peripheral tissues
- Substrates for catecholamine metabolism found in foods (tyramine) can cause a drug/food interaction
- Inhibited by class of antidepressants called MAO inhibitors

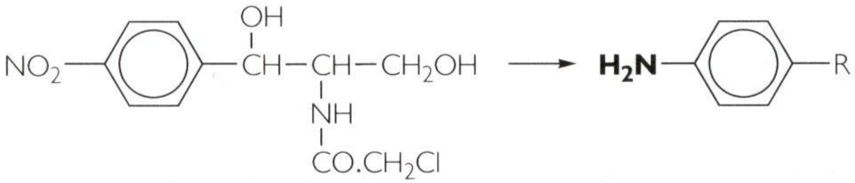
  (Inhibition of MAO isoforms in the CNS also effects levels of serotonin - Tranylcypromine)

Реакции восстановления в процессах биотрансформации (редуктазные)

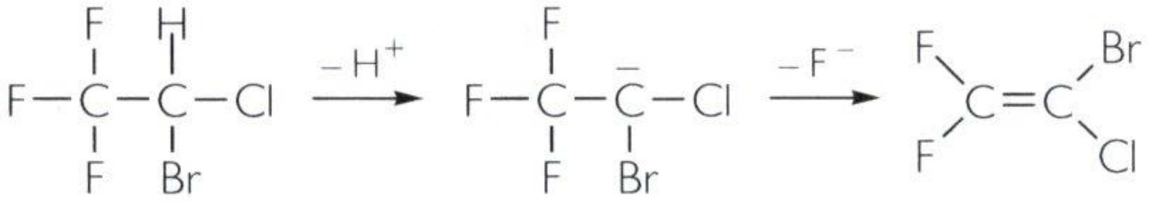
Azo-reduction:



Nitro-reduction:



Dehalogenation:

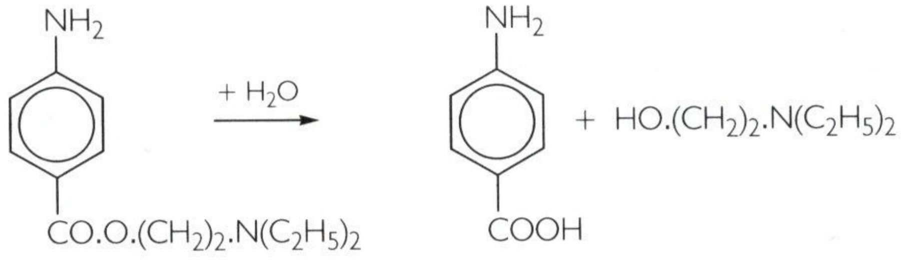


Reductive defluorination of halothane.

Гидролазные реакции в биотрансформации

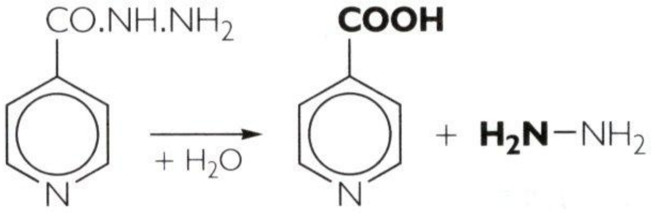
Hydrolysis reactions

Ester hydrolysis:



Hydrolysis of procaine.

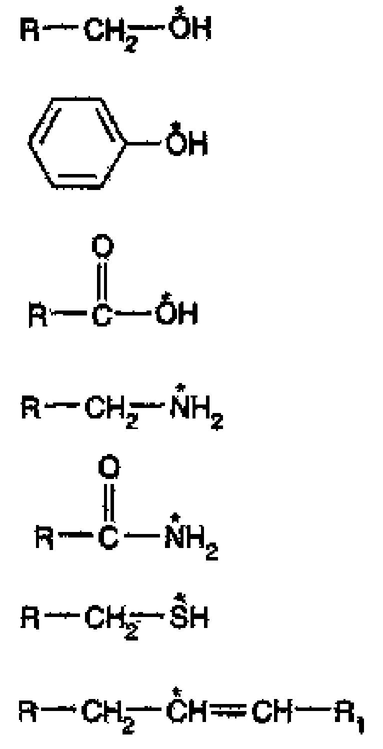
Amide hydrolysis:



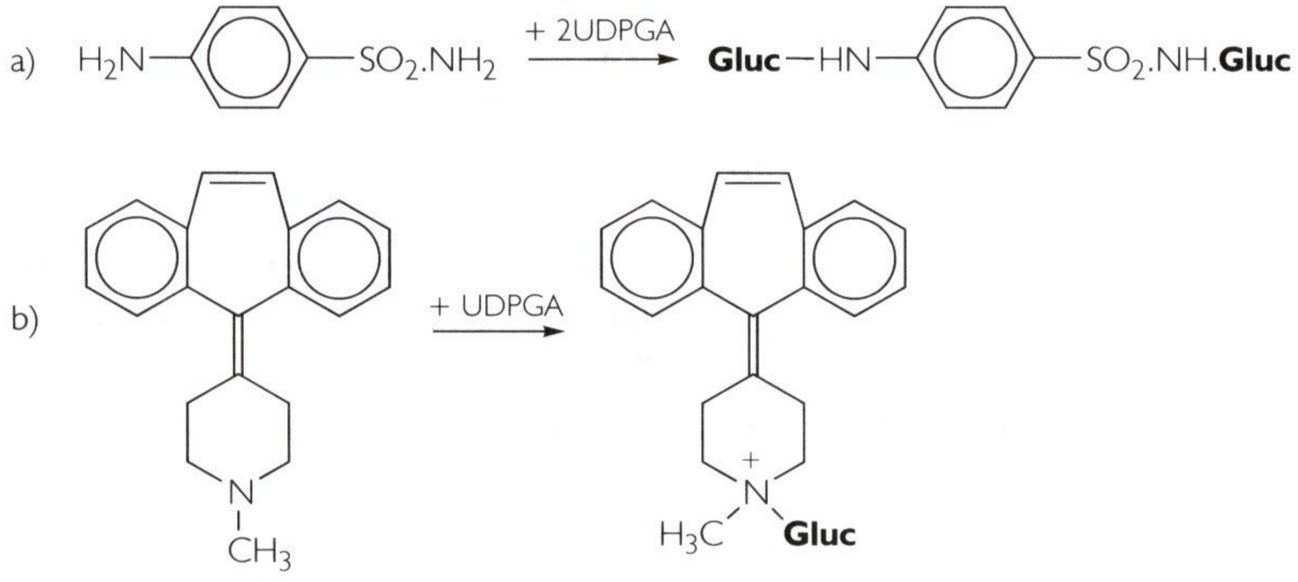
Hydrolysis of isoniazid.

Вторая фаза биотрансформации: реакции конъюгации

Типы глюкуронидов

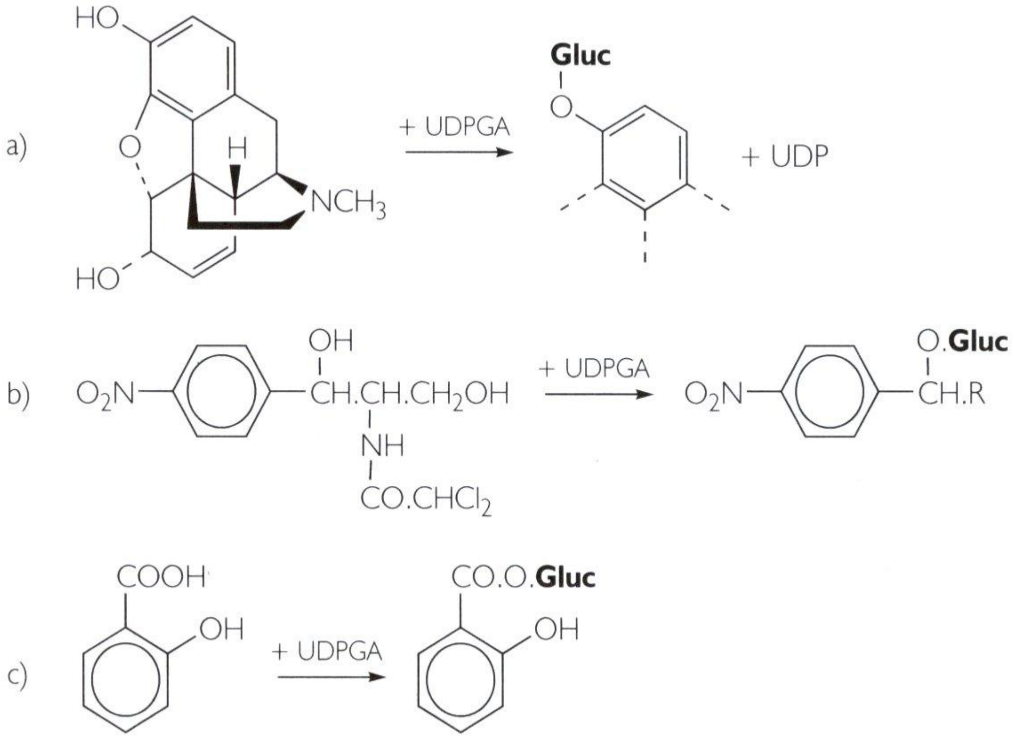
Glucuronide Type	**Субстрат**	**Пример**
O-glucuronide		Phenacetin Phenol Salicylates
N-glucuronide		Aniline Meprobamate
S-glucuronide		Methimazole
C-glucuronide		Phenylbutazone

- N-glucuronidation:
  - Occurs with amines (mainly aromatic)
  - Occurs with amides and sulfonamides



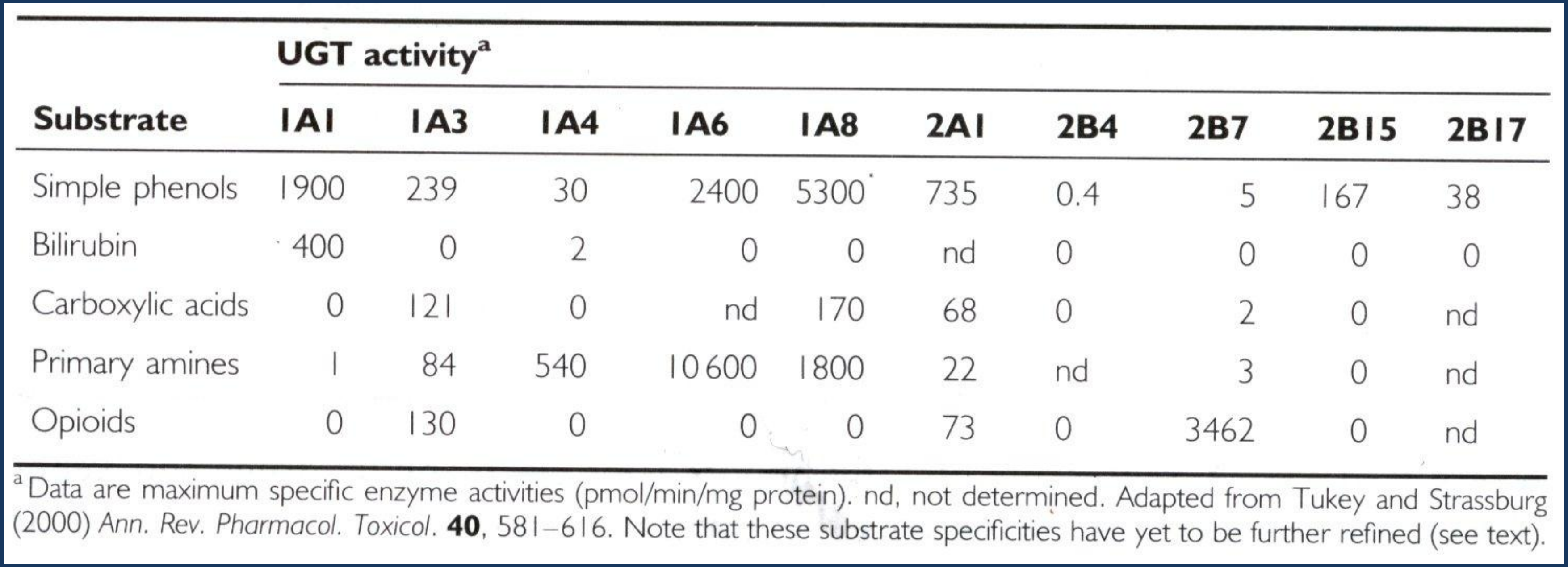
The glucuronidation of (a) sulfanilamide and (b) cyproheptidine.

- O-glucuronidation:
  - Occurs by ester linkages with carboxylic acids
  - Occurs by ether linkages with phenols and alcohols



The glucuronidation of (a) morphine, (b) chloramphenicol and (c) salicylic acid.

Субстратная специфичность глюкуронилтрансфераз



	UGT activity[a]									
Substrate	**1A1**	**1A3**	**1A4**	**1A6**	**1A8**	**2A1**	**2B4**	**2B7**	**2B15**	**2B17**
Simple phenols	1900	239	30	2400	5300	735	0.4	5	167	38
Bilirubin	400	0	2	0	0	nd	0	0	0	0
Carboxylic acids	0	121	0	nd	170	68	0	2	0	nd
Primary amines	1	84	540	10 600	1800	22	nd	3	0	nd
Opioids	0	130	0	0	0	73	0	3462	0	nd

[a] Data are maximum specific enzyme activities (pmol/min/mg protein). nd, not determined. Adapted from Tukey and Strassburg (2000) *Ann. Rev. Pharmacol. Toxicol.* **40**, 581–616. Note that these substrate specificities have yet to be further refined (see text).

Аминоацильная и пептидильная конъюгация

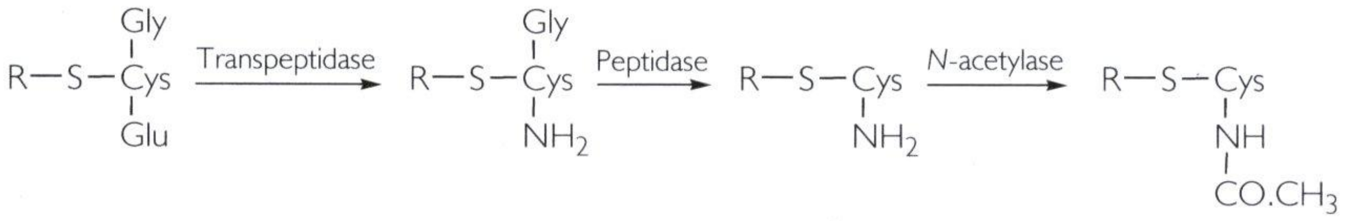
Amino Acid Conjugation:

- ATP-dependent acid:CoA ligase forms active CoA-amino acid conjugates which then react with drugs by N-Acetylation:

  Usual amino acids involved are: Gly. Gln, Ornithine, Arg

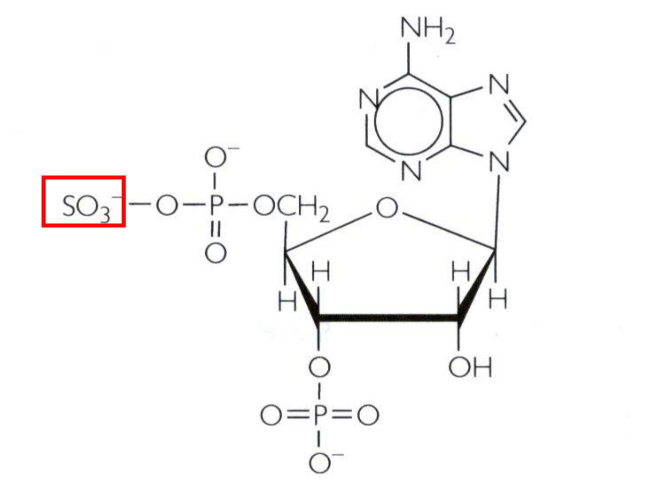
  Glutathione Conjugation:

- Tripeptide Gly-Cys-Glu; conjugated by glutathione-S-transferase (GST)
- Conjugated compounds can subsequently be attacked by g-glutamyltranspeptidase to yield the cysteine conjugate => product can be further acetylated to N-acetylcysteine conjugate



The further metabolism of a glutathione conjugate.

Конъюгация через сульфатирование



◆ Major pathway for phenols
 but also occurs for alcohols,
amines and thiols
Energy rich donor required:
PAPS (3’-Phosphoadenosine-5’-phosphosulfate)

Sulfation and glucuronidation are competing pathways:

- Sulfation predominates at low substrate concentrations
- Glucuronidation predominates at higher concentrations
- There is relatively less PAPS in cell cytosol compared to UDPGA

Sulfotransferases (=SULTs) catalyze transfer of sulfate to substrates:

- Phenol, alcohol and arylamine sulfotransferases are fairly non-specific
- Steroid sulfotransferases are very specific

Конъюгация через ацилирование

Acetylation:

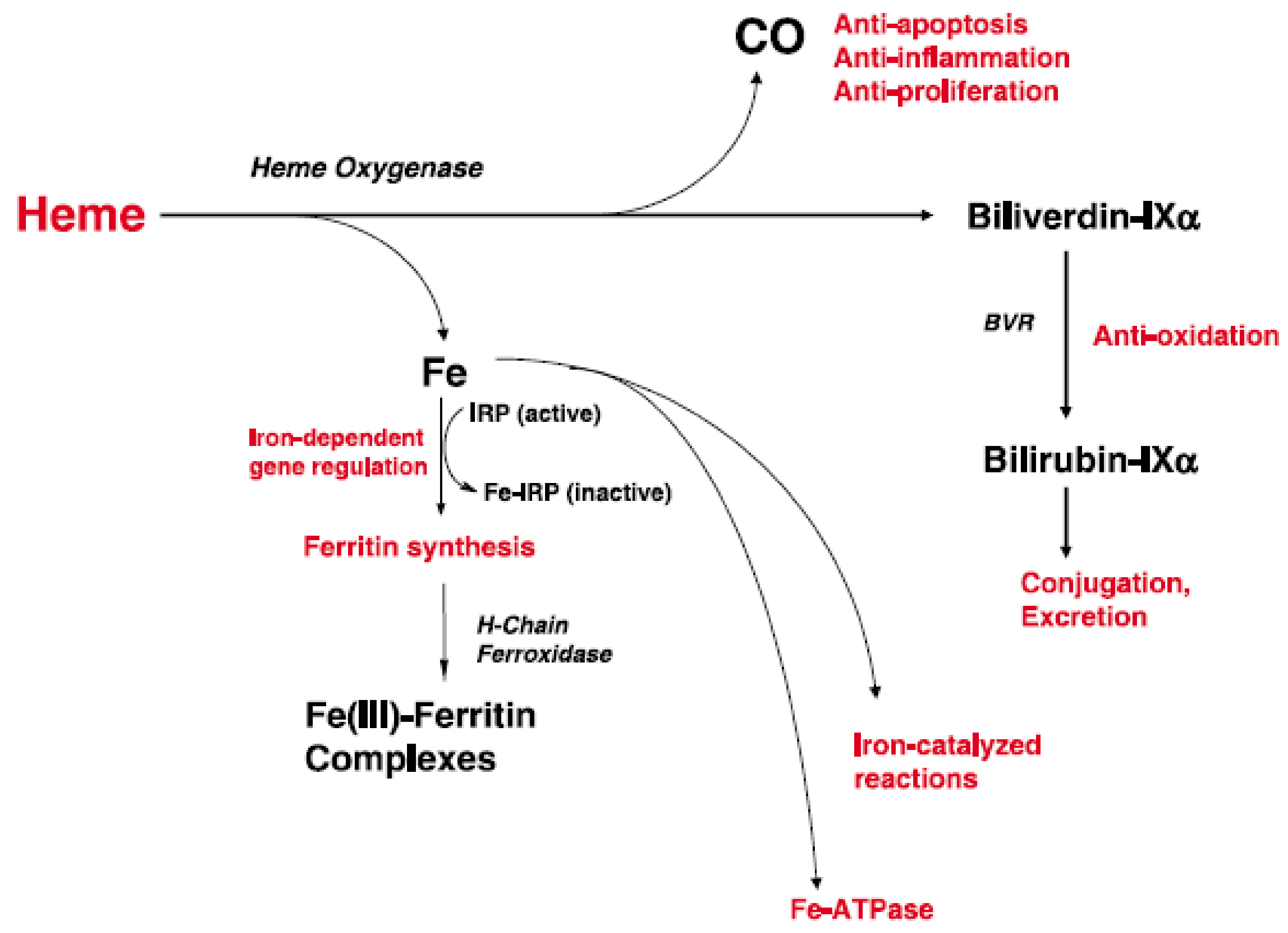
- Common reaction for aromatic amines and sulfonamides
- Requires co-factor acetyl-CoA
- Responsible enzyme is N-acetyltransferase
- Takes place mainly in the liver
- Important in sulfonamide metabolism because acetyl-sulfonamides are less soluble than the parent compound and may cause renal toxicity due to precipitation in the kidney

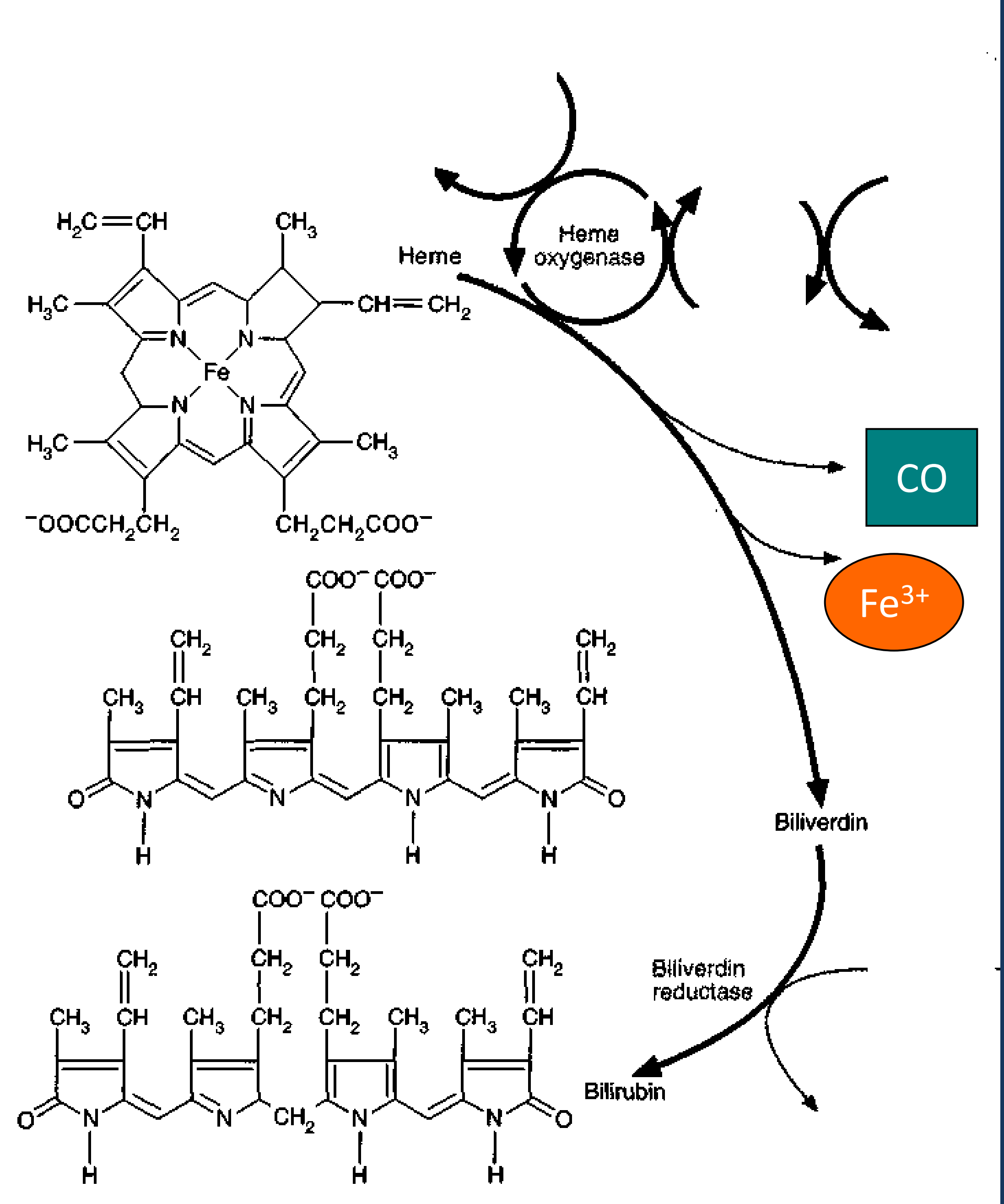
Fatty Acid Conjugation:

- Stearic and palmitic acids are conjugated to drug by esterification reaction
- Occurs in liver microsomal fraction

(Cannabinols are metabolized in this fashion => long half-life)

Биотрансформация: деградация гема





Этапы синтеза билирубина из гема: гем-оксигеназная реакция и восстановление биливердина

Глюкуронидазная реакция с билирубином